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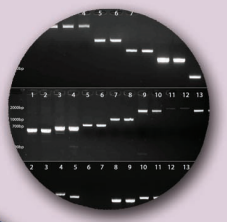
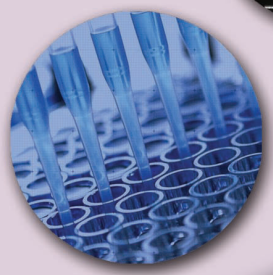
PhytoFuRa Publication No. I

Technologies

for Phytophthora, Fusarium and Ralstonia Management





PhytoFuRa
AN ICAR OUTREACH PROJECT ON
Phytophthora, Fusarium & Ralstonia

Outreach Project on
Phytophthora, Fusarium & Ralstonia
Diseases of Horticultural and Field Crops

Technologies for *Phytophthora, Fusarium* and *Ralstonia* Management

PhytoFuRa

Outreach Project on
Phytophthora, Fusarium and *Ralstonia*
Diseases of Horticultural and Field Crops (PhytoFuRA)

ICAR - Indian Institute of Spices Research
(Two times winner of Sardar Patel Outstanding ICAR Institution Award)
Kozhikode, Kerala, India

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PREFACE

Wilt pathogens *viz*., the oomycete pathogen - *Phytophthora* spp., the fungal pathogen - *Fusarium* spp. and the bacterial wilt pathogen - *Ralstonia solanacearum*, have an enormous impact on the national economy. They cause havocs to a number of horticultural and field crops of the country ranging from vegetables, fruits, spices, plantation crops, ornamentals, pulses and oil seeds. On the other hand our understanding of these pathogens has tremendously improved with the advances in sequencing and genomic techniques. To harness the power of these new generation technologies and to bring in more synergy in our efforts to deal with these pathogens, Indian Council of Agricultural Research (ICAR) launched an Outreach Project on *Phytophthora*, *Fusarium* and *Ralstonia* Diseases of Horticultural and Field Crops (PhytoFuRa) in the year 2009. Research was carried out under six thematic areas - biodiversity, diagnostics, epidemiology, genomics, host resistance, disease management and HRD. The project was operational initially in 17 centres distributed in ten states with ICAR - Indian Institute of Spices Research (ICAR-IISR) as the lead centre and subsequently during 12th Five Year Plan, three more centres were added.

The PhytoFuRa Project was one of the flagship programme of ICAR with a total outlay of ₹ 34.1 crores (2009 - 2017). It dealt with a wide range of crops including apple, banana, black pepper, brinjal, chickpea, chilli, citrus, coconut, cocoa, colocasia, ginger, guava, oil seeds, pigeon pea, potato, rubber, safflower, seed spices, sugarcane and tomato.The inter-institutional collaboration under this project was unique and exemplary. The PhytoFuRa portal (http://www.phytofura.net.in) facilitated better and faster interaction and sharing of resources among the investigators and research fellows. All this culminated in rolling out several effective control and management measures to combat these dreaded pathogens in the field. Several biocontrol agents, botanicals and other non-chemical methods for managing these pathogens were identified and were tested under field conditions.Some of these have tremendous commercial potential and can be scaled up by interested entrepreneurs. In this publication we have tried to compile such technologies that have field level application.

We take this opportunity to salute the stewardship shown by Dr. M. Anandaraj in ably leading the project since its inception and brilliantly coordinating more than 80 scientists across the country. We thank all the investigators of the project for their commendable contribution. We would like to place on record our sincere gratitude to Dr. S. Ayyappan (Former Secretary, DARE & Director General, ICAR) and Dr. Trilochan Mohapatra, the present incumbent for their valuable guidance and keen interest in the project. The support and guidance received from former Deputy Director Generals (Horticultural Science) - Dr. H.P. Singh and Dr. N.K. Krishna Kumar and Dr. A.K. Singh, the present Deputy Director General (HS) are gratefully acknowledged.The financial support for the project received from ICAR is gratefully acknowledged.

Editors

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FOREWORD

The pathogens, viz. Phytophthora spp., Fusarium spp. and Ralstonia solanacearum, cause serious diseases in a number of agri-horticultural crops leading to enormous economic losses in India. Our understanding of the host plant-pathogen interactions and other associated factors are crucial for developing sound disease management strategies. Fostering meaningful collaborations and pooling of resources in research programmes and making full use of frontier technologies is the need of the hour for enhancing efficiency. Realizing this, the Indian Council of Agricultural Research (ICAR) has supported a national level Outreach Project on Phytophthora, Fusarium and Ralstonia Diseases of Horticultural and Field Crops (PhytoFuRA) during 2009-17 at 19 Centres across the country covering six thematic areas viz. biodiversity, diagnostics, epidemiology, genomics, host resistance, disease management and the human resource development.

The project has immensely contributed to our understanding of the biology, diversity and ecology of these three pathogens of immense economic significance at national level. The concerted efforts of the collaborating institutions have largely helped in rolling out several effective field management strategies for diseases caused by these pathogens in a number of agri-horticultural crops. In addition to standardization of management technologies, the project has also accomplished several milestones of high academic value.

I am glad that the ICAR-Indian Institute of Spices Research, Kozhikode, the lead centre, has compiled and brought out three crisp publications covering technologies, protocols and scientific publications that reflect the achievement of this high impact project for the benefit of stakeholders. I take this opportunity to appreciate the efforts made by the scientists associated with planning, execution, monitoring and evaluation of the project which led to such significant outcomes. I wish that the outcome presented in this compilation will suitably be scaled up at field and help in fine tuning further research in this sector.

New Delhi
21.11.2017

(Anand Kumar Singh)

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1

Rhamnolipid bio-formulation for late blight management

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Rhamnolipids are a class of glycolipids produced by *Pseudomonas aeruginosa,* frequently cited as the well characterized among the bacterial surfactants. Rhamnolipids have been reported to posses antimicrobial properties and are reported to be inhibitory to a broad spectrum of bacteria and fungi. The antimicrobial activity of rhamnolipids provides a fitness advantage to *P. aeruginosa* by excluding other microorganisms from the colonized niche. Rhamnolipids have also been proved to be antiviral and zoosporicidal.

Summary
A number of microbes were characterized for their bio-surfactant properties and found five isolates of *P. aeruginosa* were found promising for bio-surfactant production. Testing of bio-surfactant efficacy against *Phytophthora infestans* was done through food poison test at different concentrations (0.1 µL/mL, 0.5 µL/mL, 1 µL/mL, 2 µL/mL and 5 µ/mL) and cent per cent radial growth inhibition was observed at all tested concentrations except at 0.1 µL/mL. TLC characterization of bio-surfactnant showed presence of glycolipids in the bio-surfactant. Analysis of crude bio-surfactant of *P. aeruginosa* was carried out by high performance liquid chromatography (HPLC) and mass spectroscopy (MS) and compounds were identified as rhamnolipid congeners based on their molecular formula and molecular weights. Rhamnolipid secreted by *P. aeruginosa* was collected by the methods described by Kim *et al.* (1997) for formulation development. The rhamnolipid-based formulation was developed in different solvents. Finally, cyclohexanone was selected. The detached leaves experiment was conducted to evaluate the phytotoxicity and biocontrol effect of rhamnolipid-based formulation on different concentrations starting from (0.0025 to 0.5%). The results revealed that phytotoxicity was not observed upto 0.25% concentration and this formulation was as effective as contact fungicides.

Application/Beneficiary
For the management of late blight of potato through non-chemical approach. Farmers and researchers will be benefitted.

Importance of the technology

Eco-friendly management of late blight.

Status of commercialization

To be commercialized.

Related publications

- Tomar Sonica, Singh BP, Khan MA, Kumar Satish, Sharma Sanjeev and Lal Mehi (2013). Identification of *Pseudomonas aeruginosa* strain producing bio-surfactant with antifungal activity against *Phytophthora infestans. Potato Journal* 40(2): 155-163.
- Tomar Sonica, Singh BP, Lal Mehi, Khan MA, Hussain Touseef, Sharma Sanjeev, Kaushik SK and Kumar Satish (2014). Screening of novel microorganisms for bio-surfactant and biocontrol activity against *Phytophthora infestans. Journal of Environmental Biology* 35(5): 893-899.

2

Development of dsRNA-based fungicide for management of potato late blight

Inventors
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RNA interference (RNAi) is a powerful approach for elucidating gene functions in a variety of organisms, including phytopathogenic fungi and is reported to possess antifungal activity. Exogenous application of RNA may provide an alternative strategy to chemical pesticides and genetically modified crops for combating agricultural pests and diseases. Based on this hypothesis, a dsRNA-based fungicide formulation was developed for late blight management in potato.

Summary
Developed an *in vivo* expression system to produce large amounts of dsRNAs encoding *Phytophthora infestans* genes in bacteria with a view to provide a practical control of late blight disease in potato. Designed the gene specific primers tailed with T7 promoter sequence and cloned into normal cloning vector pTZ57R/T. Positive clones were subsequently subjected to induce synthesis of dsRNA in T7 expressed cells using IPTG. IPTG induced bacterial dsRNAs promoted specific interference with the *Phytophthora* infection in potato. Furthermore, it is easy to obtain crude extracts of bacterially expressed dsRNAs which are equally effective protecting potato against *P. infestans* infections when sprayed onto plant surfaces along with emulsifying agents by a simple procedure. Late blight symptoms were significantly lower when plants were sprayed with bacterial lysate before pathogen inoculation.

Application/Beneficiary
dsRNA-based fungicide formulation for management of late blight.

Importance of the technology
Eco-friendly management of late blight.

Status of commercialization
Applied for patent.

PhytoFuRa

3

Integrated disease management schedule with fenamidone tolerant *Trichoderma harzianum* for crossandra wilt caused by *Phytophthora nicotianae*

Inventors
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Integrated disease management strategy encompasses deployment of efficacious bioagents in conjunction with judicious application of plant protection chemicals. In agricultural ecosystems, many a times this strategy seldom goes hand-in-hand due to the sensitivity of bioagents like *Trichoderma* to commonly recommended fungicides as it consequently reduces survivability and natural antagonistic activity. However, *Trichoderma* species tolerant to field concentrations of chemical fungicides due to its capability to degrade xenobiotic compounds have been reported. In this technology, an isolate of *Trichoderma harzianum* has been identified with tolerance to fenamidone which could be further used in combination to manage crossandra wilt.

Summary
The biological control agent *Trichoderma harzianum* (isolate GJ16B) that had been earlier identified as tolerant to carbendazim was tested for its tolerance to fenamidone used for the management of oomycetes. It was found to be tolerant to fenamidone also upto 200 ppm. *Trichoderma harzianum* GJ16B along with fenamidone at 0.1% can be used for the management of wilt disease in crossandra.

Application/Beneficiary
It can be used for the management of crossandra wilt disease caused by *Phytophthora nicotianae.*

Importance of the technology
It helps in the integrated disease management and can be expanded to other diseases caused by *Phytophthora.*

Status of commercialization
Commercialized by ICAR-NBAIR as carbendazim tolerant *Trichoderma harzianum* and two firms have purchased it.

4

Endophytic fungi from black pepper as biocontrol agents against *Phytophthora capsici* and *Radopholus similis*

Inventors
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Endophytic microorganisms have received increased attention due to their ability to stimulate plant defense mechanisms thereby increasing host fitness in adverse ecological niches. Anti-pathogen protection mediated through endophytes has been reported in a spectrum of plant species. In the present study, endophytic fungi isolated from black pepper have been proved to be inhibitory to *Phytophthora* and *Radopholus similis* under *in vitro* and *in planta* conditions.

Summary
Endophytic fungal isolates, both sporulating and non-sporulating types, were isolated from roots, stem and leaves of black pepper varieties, Sreekara, Subhakara, Panniyur 1 and Panniyur 3 using malt extract agar as a suitable medium. The endocarp of black pepper seeds and *in vitro* grown seedlings were found free from endophytic fungi. The common genera of sporulating endophytic fungi were *Fusarium, Colletotrichum, Penicillium, Aspergillus, Cladosporium* and *Rhizoctonia*. The non-sporulating isolates were *Diaporthe* sp., *Annulohypoxylon nitens, Daldinia eschscholzii, Ceriporia lacerata* and *Phomopsis* sp. Nine endophytic fungi isolated from black pepper *viz*., *Diaporthe* sp. (BPEF11), *A. nitens* (BPEF25, BPEF38), *D. eschscholtzii* (BPEF41, BPEF73), *Fusarium* spp. (BPEF72, BPEF75), *C. lacerata* (BPEF81) and *Phomopsis* sp. (BPEF83) showed >70% mycelial inhibition against *P. capsici* indicating their antagonistic potential. The most common anti-oomycete mechanism observed was substrate competition followed by antibiosis.
The nematicidal activity of metabolites (ethyl acetate extract) from endophytic fungi *viz., Diaporthe* sp. BPEF11(KF219919), *D. eschscholtzii* BPEF73 (KF151849) and *Fusarium* sp. BPEF75 (KF151850) were high against *Radopholus similis*. This work constitutes the first report on antifungal and antinematicidal potential of endophytic fungi inhabiting black pepper.

Application/Beneficiary

Farmers engaged in black pepper farming for eco-friendly management of major diseases.

Importance of the technology

The promising isolates could be exploited as components in biocontrol strategies in managing foot rot and slow decline diseases of black pepper.

Status of commercialization

Suitable for commercialization after validation under field trials.

Related publication

- Sreeja K, Anandaraj M and Suseela Bhai R (2016). *In vitro* evaluation of fungal endophytes of black pepper against *Phytophthora capsici* and *Radopholus similis*. *Journal of Spices and Aromatic Crops* 25(2): 113-122.

5

Talc-based bio-formulation of *Trichoderma harzianum* strain NRCFBA-44 for management of *Phytophthora* root rot in citrus

Inventors
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The role of *Trichoderma* is of immense importance in agriculture as well as environment due to its intrinsic multifarious activities including plant growth promotion and biocontrol potential. As *Trichoderma* is an eco-friendly biological control agent (BCA), it is indispensible to identify an ideal carrier material for subsequent delivery, facilitating commercialization. A suitable carrier medium is thus of utmost priority for commercial purpose as well as for field application.

Summary
A talc-based formulation of a native strain of *Trichoderma harzianum* (NRCFBA-44) isolated from Vidarbha region of Maharashtra was developed for the management of Phytophthora root rot of citrus. Briefly, fungal mycelial discs (6 mm diameter) were inoculated in 100 mL potato dextrose broth. Conidiation was estimated after seven days and the mycelial mat along with conidia from potato dextrose broth were mixed thoroughly with autoclaved talcum powder pre-treated with 0.5% carboxy methyl cellulose. The mixture was air dried in a laminar flow hood and the colony forming units were counted, subsequently packed in a polybag and sealed. The sterilized talc-based bio-formulation, containing minimum population of 10^7 cfu/g, can be stored upto eight months without significant reduction in viability. In orchards: apply 100 g of formulation/mature plant with carrier material of 1 kg well decomposed farmyard manure. In nurseries: apply 20 g of formulation/polybag of 2 L capacity. Time of usage: Two applications per annum with the onset of monsoon (June-July) and during post-monsoon (September-October) periods.

Application/Beneficiary
This formulation can be used for integrated disease management (IDM) programme for Phytophthora root rot of citrus as well as in organic citrus cultivation.

Importance of the technology
This product is useful for the eco-friendly management of *Phytophthora* root rot

disease of citrus. It also promotes growth of citrus plants.

Status of commercialization

To be commercialized.

Related publications

- Das AK, Ashok Kumar, Nerkar S and Bawage S (2012). *Trichoderma harzianum* strain NRCfBA-44, a novel biocontrol agent for the management of Phytophthora root rot in citrus. Paper presented in 5th Indian Horticulture Congress (6th-9th November, 2012).

- Das AK, Kumar A, Nerkar S, Thakre N and Landge P (2015). *Trichoderma harzianum* native strain NRCfBA-44: Isolation, characterization and formulation development for biological control of Phytophthora root rot of citrus. National Symposium on Sustainable Citrus Production: Way Forward. (27th-29th November, 2015). pp. 84-85.

- Das AK, Kumar A, Nerkar S and Thakre N (2016). Biological control of Phytophthora root rot and gummosis of citrus with novel *Trichoderma* strains native to Vidarbha region of central India. 14th International *Trichoderma* and *Gliocladium* Workshop-Principles to Practice (27th-30th November, 2016). pp. 31-32.

6

Trichoderma coir pith cake and cup formulation (TCPC) for management of bud rot disease of coconut, stem canker and seedling blight diseases of cocoa

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Low cost formulations utilizing locally available substrates are necessary for wide adoption of biocontrol formulations to manage plant diseases. At ICAR-CPCRI, a low cost technology on *Trichoderma* formulation has been developed for increased shelf life and easy application.

Summary
A technology was developed to prepare *Trichoderma* formulation *viz.*, *Trichoderma* coir pith cake and cup (TCPC) using coir pith, maida flour and *Trichoderma harzianum* (CPCRI TR 28). To prepare the formulation as a solid cake, 30 g mixture (coir pith, maida and *T. harzianum*) was compressed manually using a kitchen press. To prepare a single solid cake, 30 g mixture was placed inside the outer case of kitchen press on a plastic sheet. Further, it was compressed using the inner cylinder to get solid cake. The cakes thus prepared were dried in an oven at 38-40°C for four days. It is also found that dried TCPC packed in polythene bags could be stored at room temperature (26-30°C) for 10 months with high level of *T. harzianum* population. Through a simple activation process of moistening the dried TCPC and incubating under room temperature for two days, luxurious growth of *Trichoderma* could be achieved.

Application/Beneficiary:
The *Trichoderma* coir pith cake formulation could be used against cocoa seedling blight and canker and bud rot of palms.

Importance of the technology
Formulation has a shelf life of more than 10 months.

All materials used are natural and completely biodegradable.

Concept of "wealth from waste" of waste management.

Reactivation of the formulation (even after 10 months) with water which re-vitalize *Trichoderma* population attaining the recommended cfu.

Low cost formulation with locally available substrates.

Status of commercialization

Applied for patent and to be commercialized.

Related publication

- Chandra Mohanan R, Prabha K Peter and Sharadraj KM (2013). Production technology of coir pith cake formulation of *Trichoderma harzianum*. *Journal of Plantation Crops* 41(2): 214-218.

7

Development and application of eco-friendly management strategy against collar rot of apple caused by *Phytophthora cactorum*

Inventors

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The most popular and effective management strategy for controlling collar rot disease of apple is through synthetic fungicides and several applications are resorted during each season. However, indiscriminate and non-judicious application of chemical fungicides invites, the so-called "3 R-problem" (resistance, resurgence and residue). Biological control with microbial antagonists is a promising alternative for synthetic fungicide usage with low environmental impact. Here, an eco-friendly management strategy against apple collar rot has been developed with three different components *viz.*, promising botanical in cow urine based-formulation, approach grafting and application of red soil.

Procedure/Summary

Formulation of cow urine decoction was prepared by incorporating three most effective botanicals namely seeds of *Melia azedarach* (10 kg), fresh leaves of *Murraya koenigii* (5 kg) and *Eucalyptus* in 50 L of cow urine and subjected to fermentation for 35 days. Thereafter, it was filtered through cheese/muslin cloth and used immediately or alternatively it was pasteurized at 58°C for 2 h for three consecutive days and stored for further use. Cow urine-based formulation was applied as soil drench thrice a year during March, June and August @7.5% (10 L/tree upto 2 feet around the stem). For approach grafting, 34 young apple plants of resistant root stocks MM111/M9 were planted at 11.5 feet around the main trunk and were approach grafted during the month of April-May. Combined application of red soil (3 feet area around stem upto 10 cm depth), cow urine decoction and approach grafting was highly effective in increasing shoot length and exhibited lesion recovery of collar portion (38.4%). Use of this technology continuously for 2-3 years resulted in complete recovery of collar rot infected apple under orchard conditions.

Application/Beneficiary

Apple growers.

Importance of the technology

Effective in controlling collar rot of apple under orchard conditions.

The technology is effective, eco-friendly and economical since all the ingredients are easily and locally available.

Status of commercialization

To be commercialized.

Related publications

- Sharma IM, Negi HS, Sharma S and Khosla K (2014). Prevalence and integrated management of collar rot of apple through eco-friendly methods. In: Proceedings of National Symposium on "Innovative and eco-friendly research approaches for plant disease management", Dr. PDKV, Akola, Maharashtra, 8th-10th January, 2014. pp. 58-59.
- Sharma IM, Negi HS and Shweta Sharma (2015). Prevalence, diagnosis and integrated management of collar rot in apple through eco-friendly methods. In: Proceedings of National Symposium on "Understanding host-pathogen interaction through science of omics." ICAR-IISR, Kozhikode, 16th-17th March, 2015. p.176.

8

A thermo-tolerant, symbiotic *Trichoderma asperellum* TaDOR 7316 WP (MTCC No. 5623) formulation for use as biocontrol agent, defense inducer and plant growth promoter

Inventors

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A thermotolerant strain of *Trichoderma asperellum* TaDOR 7316 was isolated from rhizosphere of soybean from Rajasthan. *T. asperellum* TaDOR 7316 at its hyphal stage can tolerate extreme temperature regimes due to accumulation of higher quantities of osmoprotectants. The strain possessed biocontrol efficacy against Fusarial wilt and Phytophthora damping off in castor and safflower.

Procedure/Summary

As seed coating agent @10 g/kg seed *T. asperellum* TaDOR 7316 will have high economic value as high crop yield can be achieved in rainfed agriculture and under drought conditions. The isolate could survive high temperature at both hyphal and sporulating stages of its life cycle. It could also retain its usual morphological characteristics after recovery from heat stress conditions.

Application/Beneficiary

Protects crops against variety of diseases and promotes plant growth. The thermotolerant strain is able to antagonize plant pathogenic fungi. It produces extracellular enzymes *viz*., chitinases, glucanases, cellulases and amylases as well as plant growth promoting molecules that help in controlling fungal diseases and enhances plant growth promotion.

Importance of the technology

Shelf life of the bioagent in powder formulation is more than 16 months at room temperature compared to six months shelf life of similar products available in the market.

It is a superior strain having broad spectrum abilities *viz*., endophytic root colonization, defense induction against wilt and root rot diseases.

Status of commercialization

Applied for patent and to be commercialized.

PhytoFuRa

9

Soil amendment of lime for bacterial wilt management in brinjal

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The practice of incorporating amendments of various categories to soil plays a pivotal role in restoring soil health and improving quality directly by influencing its physio-chemical properties and proliferation of resident soil microbiota. Soil amendments are also reported to be effective against several soil inhabiting pathogens by creating a hostile environment for its survival, favouring beneficial microbes and improving plant health. Here, the technology highlights the importance of lime as soil amendment in managing bacterial wilt of brinjal.

Procedure/Summary

Mix the required quantity of lime (@10 and 20 t/ha) with soil upto a depth of 10-15 cm. Irrigate the soil twice in 15-20 days and plant brinjal seedlings.

Application/Beneficiary

A pre-plant measure to minimize bacterial wilt in brinjal.

Importance of the technology

Simple soil amendment to reduce bacterial wilt.
Can be combined with biocontrol application.
Improves general soil conditions by increasing soil pH.

Status of commercialization

To be commercialized.

10

Grafting - A novel technology for multiple disease management in tomato

Inventors

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Grafting is a widely adopted horticultural practice in which a root stock and scion are united for several advantages. It is considered as a sustainable and viable technology to combat several diseases incited by soil-borne plant pathogens using resistant root stocks, thus indirectly imparting resistance to the susceptible cultivars with desirable traits. In this technology, grafting has been used as a strategy for multiple disease management in tomato.

Procedure/Summary

A brinjal root stock (EG219-*Solanum melongena*) of 30 days old resistant to Fusarial wilt, bacterial wilt and nematode (imported from AVRDC, Taiwan) was used to make grafted plant with tomato (cultivar Kashi Aman resistant to *Tomato leaf curl virus*) scion from 20 days old seedling. These grafted plants were evaluated under field conditions for their performance. Grafted plants were found resistant to Fusarial wilt, root knot nematode and *Tomato leaf curl virus* compared to non-grafted plants.

Application/Beneficiary

Useful to manage multiple diseases such as Fusarial wilt, root knot nematode and tomato leaf curl disease in tomato under field conditions.

Importance of the technology

Source of multiple disease resistance in grafted tomato plants.

Status of commercialization

To be commercialized.

PhytoFuRa

11

Integrated disease management schedule for the wilt in gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* using carbendazim tolerant *Trichoderma harzianum*

Inventor
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Exploiting the chemo-tolerant trait of potential biocontrol agents provides a platform for combined application of potent anti-microbial agents along with synthetic molecules with an outlook to subjugate the targeted pathogens both biologically and chemically. In this technology, an isolate of *Trichoderma harzianum* tolerant to carbendazim, a commonly recommended fungicide, has been identified to manage wilt disease of gladiolus.

Procedure/Summary

Trichoderma harzianum (isolate GJ16B) was found to be tolerant to carbendazim and other benzimidazoles (cross resistance) upto 1000 ppm. *T. harzianum* GJ16B along with carbendazim at 0.1% can be used to manage wilt disease of gladiolus.

Application/Beneficiary

It can be used for the management of wilt disease of gladiolus.

Importance of the technology

It helps in the integrated disease management of gladiolus wilt and can be expanded to other wilt diseases.

Status of commercialization

Commercialized by ICAR-NBAIR as carbendazim tolerant *Trichoderma harzianum* and two firms have already purchased it.

12

Management of bacterial wilt in ginger using apoplastic bacteria - *Bacillus licheniformis*

Inventors

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Endophytes confer protection to host plants against pathogens by triggering defense pathways, secreting anti-microbial metabolites or through competition for colonizable niches and nutrients. Suppression of plant diseases due to the action of endophytic microorganisms has been demonstrated in several host-pathosystems. In ginger, apoplastic bacteria were isolated and evaluated for *in vitro* and *in planta* inhibitory activities against *Ralstonia solanacearum* of which IISR GAB 107 was found promising.

Procedure/Summary

IISR GAB 107 showed significant reduction in bacterial wilt incidence (66.42%) compared with control under greenhouse challenge inoculated conditions and 100% disease reduction could be attained under field conditions in sick plots. Seed treatment and soil application of IISR GAB 107 were proved effective in managing the bacterial wilt.

IISR GAB 107 treated ginger plots

1. Select an area and test for soil pH and for the presence *R. solanacearum* race 4 biovar 3. Prepare beds (February-March) and solarize them for 40-50 days using polythene sheet of gauge 100 µ (preferably 1st March to second week of April).
2. Treat rhizomes/seeds with IISR GAB 107 @10^8cfu/g by soaking seed rhizomes in bacterial suspension for 1 h before planting in either conventional or protray method (@2 kg talc formulation in 100 L water and soak the seeds for 1 h before planting).
3. Apply IISR GAB 107 at the time of planting and at 30, 45 and 60 days (4 applications @5 L/bed having a cfu of 10^8/ml *i.e.* 1 kg talc formulation in 100 L water and drench @5 L/bed). Other agronomic practices may be adopted as recommended in package of practices of ICAR-IISR.

Importance of the technology

Highly effective in controlling bacterial wilt under field conditions.

Application/Beneficiary

Ginger growers.

Status of Commercialization

To be commercialized.

Related publication

- Prameela TP and Suseela Bhai R (2016). Apoplastic bacteria from ginger - A prelude to the biological control of *Ralstonia solanacearum* race 4 biovar 3. In: Khetarpal RK, Kalyan K Mondal, Dubey SC, Rao GP, Celia Chalam, V Namrata Singh, Deeba Kamil, Bishnu Maya Bashyal , Prashant P. Jambhulkar, Prakash G, Dinesh Singh and Pratibha Sharma. (Eds.) IPS 6th International Conference on "Plant Pathogens and People" (23rd-27th February, 2016). p. 676

13

Technology for the management of bacterial wilt in ginger using calcium chloride

Inventors

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Various non-pesticide chemicals have the potential to manage bacterial wilt under field conditions and they have less deleterious effects on the environment. Expensive chemicals and repeated applications are only possible for commercial crops which might incur substantial economic losses, if recommended measures are not executed in time. Studies conducted at ICAR-IISR revealed that soil application of calcium chloride (fused) is promising against bacterial wilt of ginger under field conditions especially in heavily infested sick plots.

Procedure/Summary

1. Select an area and test for soil pH and for the presence *Ralstonia solanacearum* race 4 biovar 3.

Calcium chloride treated ginger plots

2. Prepare beds and solarize them for 40-50 days using polythene sheet of 100 µ gauge (preferably 1st March to second week of April).
3. Apply calcium chloride (3%) at the time of planting and at 30, 45 and 60 days (4 applications @5 L/bed).
4. Adopt other agronomic practices as recommended in package of practices of ICAR-IISR.

Importance of the technology

Highly promising in controlling bacterial wilt under field conditions.

Application/Beneficiary

Ginger growers.

Status of commercialization

To be commercialized.

14

Bacteriophages as potential antagonists against *Ralstonia solanacearum* race 4 biovar 3

Inventors

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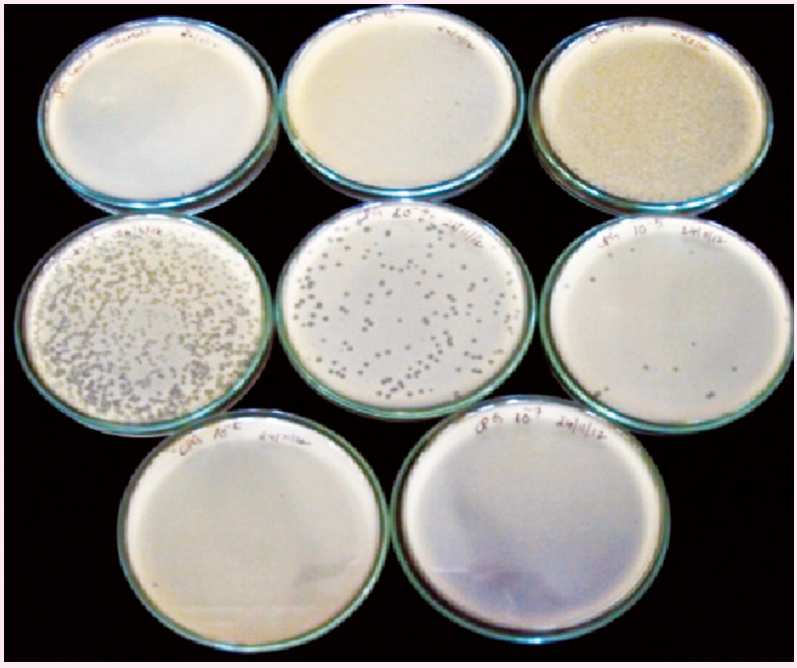
Phage therapy or phage biocontrol is an innovative method of biological control. The advantage of phage therapy is the reduction in the use of chemical agents thereby avoiding problems associated with environmental pollution, ecosystem disruption and pesticide residues. Phage therapy in agricultural settings was extensively explored as means of controlling plant pathogens. In the present investigation, lytic phages were isolated from ginger growing fields and evaluated for biocontrol potential against bacterial wilt pathogen of ginger.



Isolation of phages

Procedure/Summary

The isolated phage (φ-1) was tested for its specificity towards twelve different *Ralstonia solanacearum* isolates from different locations. The phage φ-1 was found to be infecting seven isolates collected from Wayanad area, but was non-infective to isolates from other places from India and also isolates from other hosts, showing its host specificity. The destruction level of *R. solanacearum* with phages, showed a 4 fold reduction. *In planta* evaluation by drenching phage suspension to 60 days old ginger plants and further challenging resulted in 30% disease reduction. In order to get higher disease reduction, the phages should outnumber the pathogen. This can be achieved using avirulent pathogen as host for multiplication of phages. This is the first report of employing phages for the biological control of *R. solanacearum* race 4 biovar 3.

Application/Beneficiary

An alternative for biological control of *R. solanacearum* race 4 biovar 3.

Importance of the technology

Bacterial wilt management in ginger.

Status of commercialization

To be commercialized.

Related publication

- Prameela TP, Suseela Bhai R and Anandaraj M (2012).Isolation of phages infecting *Ralstonia solanacearum* causing bacterial wilt in ginger (*Zingiber officinale* Rosc.). Paper presented in Indian Phytopathological Society (South Zone) Conference, 16th-17th November, 2012. ICAR-SBI, Coimabtore.

15

Bioloiological control of bacterial wilt in brinjal using endophytic and rhizobacteria

Inventors

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The bacteria isolated from rhizosphere of several plant species have been proved to be beneficial directly having an effect on nitrogen fixation, nutrient solubilization and indirectly by antagonizing pathogens by the production of siderophores, 1,3-glucanase, antibiotics etc. Similarly, bacterial endophytes are able to colonize the ecological niche similar to that of plant pathogens, especially vascular wilt pathogens. Here, a talc-based formulation of promising antagonistic rhizospheric and endophytic bacteria was developed and the method of mass multiplication and formulation was standardized.

Procedure/Summary

Best disease control is achieved when the biocontrol agents are applied in nursery (@50 g/m^2) and in the main field (1.25-2.5 g/plant) during transplanting. Apply 50 g of the talc-based formulation per m^2 of the nursery area before sowing the seeds. Treat seeds with talc formulations @2 g per 100 g seeds. Drench seedlings in the nursery with the bioagent suspension (20 g/L of water) after 20 days. During planting time, mix talc formulation of the biocontrol agent containing 10^8 cfu/g with water (25-50 g/L) to form a suspension and dip the seedlings in the suspension. After transplanting, add about 50 ml of the suspension per plant. Repeat the same application after 15-20 days of transplanting.

Application/Beneficiary

Nursery and main field application is recommended for bacterial wilt management and to increase the yield in brinjal.

Importance of the technology:

In the field evaluation and demonstrations, the biocontrol treatments recorded less disease and higher yield.

Eco-friendly method of bacterial wilt management.

Status of commercialization

To be commercialized.

PhytoFuRa

16

Xylem residing bacteria (XRB) and 3OH-PAME degrading bacteria for biocontrol of bacterial wilt

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Published literature highlights the role of endophytic bacteria as biocontrol agents against several plant pathogens. The capability of colonizing internal host tissues and ability to produce volatile and diffusible molecules that inhibit pathogen, induction of systemic resistance and directly or indirectly promoting plant growth have made endophytes a valuable tool in agriculture to improve crop performance. The wilt prevention ability of xylem residing bacteria of solancaeous crops that share an ecological niche with the bacterial wilt pathogen was explored. Here, xylem residing bacteria were isolated and assessed for their biocontrol activities against *Ralstonia solanacearum* in brinjal.

Procedure/Summary

Promising strains of endophytic bacteria were formulated using talc. Add 50 g of the talc-based formulation per m^2 area of the nursery before sowing the seeds. Treat the seeds also with talc formulations @2 g per 100 g seeds. Drench seedlings in the nursery with the bioagent suspension (20 g/L of water) after 20 days. During planting time, mix talc formulation of the biocontrol agent containing 10^8 cfu/g with water (50 g/L) to form suspension and drench seedlings with 50 ml of the suspension. Repeat the same application after 15-20 days of transplanting.

Application/Beneficiary

Nursery and main field application is recommended for bacterial wilt management and increased yield.

Importance of the technology

XRB colonized the plants, improved growth and reduced bacterial wilt incidence.

Quorum quenching mechanism by few XRB isolates adds the unique mechanism of action.

This study demonstrates the potential use of endophytic bacteria as quorum quenching biocontrol agents for management of bacterial wilt in brinjal.

Status of commercialization

To be commercialized.

17

Grafting of cultivated brinjal on wild brinjal - A promising technology to manage bacterial wilt

Inventors

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Grafting brinjal on resistant root stocks has been reported to be an effective control measure against wilt diseases. The practice of grafting brinjal on *Solanum torvum* is extensively adopted in vegetable industry in Japan. In the present work, *S. torvum* was evaluated as a root stock in brinjal to manage bacterial wilt incidence and thereby realizing higher yield levels.

Procedure/Summary

Sow seeds of *S. torvum* (bacterial wilt resistant root stock) in nursery beds. Transplant about 5-6 cm tall seedlings into nursery bags containing potting mixture and select 45-50 days old seedlings (stem: 3 mm diameter) for making grafts. Sow the seeds of susceptible varieties in nursery beds/trays. Select 30-35 days old seedlings (stem: 3 mm diameter) for preparing the grafts using the top wedge method. Tie the graft union with thin poly sheet (50-100 µ) to keep the union intact till the tissues are healed. Maintain the grafted plants in high humidity chambers with less direct sunlight for 8-10 days and until the new leaf emergence is noticed. Harden the grafts at outdoors for 3-4 days and the hardened grafts can be used for field planting.

Application/Beneficiary

Grafting of susceptible cultivated brinjal on resistant root stock is a simple technique to manage bacterial wilt, without any reduction in yield.

Importance of the technology

Simple technology for managing bacterial wilt of brinjal.

Higher disease control efficiency.

The plants do not allow the pathogen to grow in their tissues resulting resistance.

Compatible with other eco-friendly management strategies.

Status of commercialization

To be commercialized.

PhytoFuRa

18

Grafting of tomato on wild brinjal and other bacterial wilt resistant root stock to reduce bacterial wilt

Inventors

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Grafting has been utilized as a viable technology to manage bacterial wilt disease of tomato worldwide and has been recently proposed in the United States for open-field and high-tunnel production systems. It is of critical importance to evaluate rootstocks that are readily available to growers for resistance to different strains of *Ralstonia solanacearum*.

Procedure/Summary

Sow seeds of *Solanum torvum* and Surya S-4 (bacterial wilt resistant root stock of wild brinjal) in nursery beds/trays. Transplant about 5-6 cm tall seedlings into nursery bags containing potting mixture. Select grown-up root stock seedlings (stem: 3 mm diameter) for making grafts. Sow seeds of bacterial wilt susceptible tomato cultivars (majority of the commercial varieties) to be used as scion material in nursery beds/trays. Select about 25-30 days old seedlings (stem: 3 mm diameter) and graft them using top wedge grafting method. Tie the graft union with thin poly sheet (50-100 µ) to keep the union intact till the tissues are healed. Maintain the grafted plants in high humidity chambers with less direct sunlight for 8-10 days until the new leaf emergence is noticed after 5-7 days. Harden the grafts at outdoors for 3-4 days and the hardened grafts may be used for field planting.

Application/Beneficiary

Grafting of susceptible tomato on bacterial wilt resistant brinjal root stock controlled bacterial wilt. This technology could be used in bacterial wilt endemic areas and also in polyhouse production systems.

Importance of the technology

Considering the non-availability of desired bacterial wilt resistant tomato varieties, grafting approach could be a promising strategy for the bacterial wilt management.

Very useful in polyhouse tomato cultivation.

Status of commercialization

To be commercialized.

19

Talc-based formulations of *Pseudomonas fluorescens* IIHRPF-24 and *Bacillus subtilis* IIHRBS-39

Inventors

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Contact : director@iihr.res.in

Commercialization of biocontrol agent-based technologies is a multi-step process involving a broad range of activities of which development of formulations and delivery systems offer great challenges. Talc-based formulations are widely adopted due to their beneficial properties such as providing appreciable shelf life period to the bioagent, easiness in delivery and application.

Procedure/Summary

Prepare formulations of potential strains of *Pseudomonas fluorescens* IIHRPf-24 and *Bacillus subtilis* IIHRBs-39 in the laboratory using talc. Grow *P. fluorescens* and *B. subtilis* cultures separately in nutrient broth for 48 h using a shaking incubator at temperature 28±2°C at 150 rpm. Add 10 g of carboxy methyl cellulose to 1 kg of sterile talc as carrier material and mix well. Mix about 400 ml of bacterial suspension containing 2.5 x 10^8 cfu/ml with the carrier material under sterile condition. Add 15 g of calcium carbonate to the formulation to adjust the pH to 7.0.

Application/Beneficiary

The talc-based formulations of *P. fluorescens* and *B. subtilis* can be used for the integrated management of bacterial wilt of tomato and brinjal.

Importance of the technology

The talc-based formulations of the bioagents are eco-friendly, cost effective and have been tested in farmer's field as one of the components of IDM package.

Status of commercialization

Toxicological data of the bioagents has to be generated for commercialization of the above products.

PhytoFuRa

20

Formulation for the storage and delivery of *Fusarium* suppressive *Trichoderma* spp.

Inventors

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Soil or liquid-based formulations should possess intrinsic qualities for enhancing shelf life of the bioagents and their compatibility. Importance should be given while selecting and standardizing substrates for facilitating easy delivery and also to make the technology cost effective and easily adoptable.

Procedure/Summary

A liquid-based formulation using inexpensive molasses was developed for the long term storage (15 months) and delivery of *Trichoderma* spp. The *in vivo* testing of the efficacy of liquid formulation of *Trichoderma* sp. NRCB3 (which was stored for 13 months at 25± 2°C) at 5, 10 and 15% concentration in cultivar Grand Naine indicated that the application of all the concentrations resulted in complete control of Fusarial wilt disease.

Application/Beneficiary

It can be used to store the bioagents for long time (upto 15 months) and can be used for drenching, in drip irrigation and also for spraying.

Importance of the technology

The materials used in this formulation are cheaper and hence cost effective.

Status of commercialization

To be commercialized

21

Management of Fusarium wilt disease using bioagents and botanicals in banana

Inventors
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Plants are considered as reservoirs of both endophytic microbes inhabiting the intercellular spaces as well as vasculature and potential antimicrobial bio-active molecules which can be exploited to manage diseases incited by various pathogenic microorganisms. Combination of various soil dwelling potential antagonists like *Trichoderma*, efficacious endophytes and plant species with antimicrobial activity has been proved to be promising in managing a broad range of plant diseases and attaining appreciable yield.

Procedure/Summary
Combined application of rice chaffy grain formulation of fungal endophyte *Penicillium pinophilum* (Bc2) + rhizospheric *Trichoderma* sp. (NRCB3) @50 g/plant and soil drenching with liquid formulation containing endophytic *Trichoderma asperellum* (Prr2) + *Bacillus flexus* (Tvpr1) @1 L/plant or soil drenching with aqueous *Zimmu* leaf extract (50%) @1 L/plant three times (at the time of planting, two months after planting and four months after planting) recorded almost complete control of the disease and increase in the yield (170.5%) under field condition (internal disease score of 1.1 as against 6.0 in the control). These treatments also increased the plant growth and yield particularly bunch weight (170.5%) significantly and 100% harvest of good bunches compared to only 35% harvest in the control plants.

Application/Beneficiary
Useful to manage the Fusarium wilt disease of banana under field conditions.

Importance of the technology
The technology developed is more effective in suppressing the disease under field condition and cost effective.

Status of commercialization
To be commercialized.

Related publications

- Gopi M and Thangavelu R (2014). Suppression of Fusarium wilt disease of banana by *Zimmu* (*Allium cepa* x *Allium sativum*) leaf extract. *African Journal of Microbiology Research* 8(31): 2904-2915.
- Thangavelu R and Gopi M (2015). Field suppression of Fusarium wilt disease in banana by the combined application of native endophytic and rhizospheric bacterial isolates possessing multiple functions. *Phytopathologia Mediterranea* 54: 241-252.
- Thangavelu R and Gopi M (2015). Combined application of native *Trichoderma* isolates possessing multiple functions for the control of Fusarium wilt disease in banana cv. Grand Naine. *Biocontrol Science and Technology* 25: 1147-1164.

22

Cost effective method for mass production of *Trichoderma*

Inventors
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Developing farmer-friendly and cost effective technologies for mass multiplication of biocontrol agents is of prime importance in the era of biological control. Exploiting low cost and naturally available substrates are indispensible for the technology to be widely adopted among the farming communities.

Procedure/Summary

A cheaper, easier and farmer-friendly method for mass production of *Trichoderma* spp. using rice chaffy grains. By employing this technology, *Trichoderma* can be mass multiplied within 5-7 days under farm condition. The colony forming units of *Trichoderma* per gram of substrate was several folds higher than the normal formulation available in the market. The same technology was also used to mass multiply entomopathogenic fungi such as *Beauveria bassiana* and *Verticillium lecanii* for effective management of insect pests.

Application/Beneficiary

For mass multiplication of *Trichoderma* and entomopathogens using cheaply available wastes like rice chaffy grains at on-farm level.

Importance of the technology

Requires less time, cost effective and useful for the mass production of biocontrol agents such as *Trichoderma* and entomopathogens like *Beauveria* and *Verticillium*.

Status of commercialization

To be commercialized.

Related publication

- Thangavelu R and Mustaffa M M (2010). A potential isolate of *Trichoderma viride* NRCB1 and its mass production for the effective management of Fusarium wilt disease in banana. *Tree and Forestry Science and Biotechnology* 4: 76-84.

PhytoFuRa

23

IDM strategy for the management of bacterial wilt of tomato and brinjal

Inventors

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ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka-560089

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Integrated disease management strategy, preferably based on the principles of biocontrol and enriching soil with natural substrates are considered as economically viable and sustainable strategies to manage various diseases. Bacterial wilt pathogens are generally soil-borne in nature and engineering soil architecture through addition of organic matter supplemented with efficacious bioagents suppress the pathogen, reducing its inoculum potential and thereby managing the disease efficiently and eco-friendly.

Procedure/Summary

An integrated strategy for the management of bacterial wilt in tomato and brinjal has been developed. The strategy includes (i) soil amendment with green manure (sunnhemp) 25 kg/ha and farmyard manure 20 t/ha (ii) followed by planting of tomato seedlings pre-treated with bioagent, *Pseudomonas fluorescens* (seed or seedling treatment 10 g/L) (iii) soil drenching around root zone with suspension of bioagent (10 g/L) twice at fortnightly intervals after twenty days of planting and (iv) application of 2-3 sprays of copper oxychloride 2 g/L at fortnightly intervals.

Application/Beneficiary

The IDM strategy is useful in the management of bacterial wilt of tomato and brinjal.

Importance of the technology

The IDM strategy is eco-friendly and cost effective and has been tested in farmer's field.

Status of commercialization

The IDM strategy is tested in farmer's field and is working well against bacterial wilt in tomato and brinjal and to be commercialized.

24

Development of bioformulations of *Bacillus amyloliquefaciens*, *Bacillus subtilis* and *Pseudomonas fluorescens*

Inventors
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Development of bio-formulations is a challenging process primarily involving identification of a substrate compatible with the bioagent. The substrate should be endowed with ideal properties especially desirable pH and moisture content so that survivability of the microbe is not adversely affected during storage.

Procedure/Summary
Inoculate one loop of individual bacterial isolates into 400 ml of autoclaved broth medium and incubate at 28±2°C on a shaker at 150 rpm for 48 h. The bacterial population in broth will be $9x10^8$ cfu per ml after 48 h growth. Take one kg of sterilized talc powder in a metal tray and adjust the pH to neutral by adding $CaCO_3$. Add 10 grams of carboxy methyl cellulose (CMC adhesive) to 1 kg talc powder before mixing. Mix 400 millilitres of 48 h grown inoculum with 1 kg of talc powder under aseptic conditions. Air dry to reduce the moisture content (less than 20%) overnight, pack and seal in polybags.

Application/Beneficiary
Bacterial and fungal disease management.

Importance of the technology
This is more effective against pathogenic bacteria and fungi.

Status of commercialization
To be commercialized.

PhytoFuRa

25

Endophytic fungus (PGPF) *Piriformospora indica* as a promising plant growth promoting agent

Inventors

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Piriformospora indica, an axenically cultivable phyto-promotional, biotrophic mutualistic root endosymbiont, has been reported to mimic capabilities of typical arbuscular mycorrhizal fungi. This fungus has the potential to colonize roots of a wide range of higher plants and provide plants multifaceted amenities.

Procedure/Summary

Mass multiply *P. indica* by culturing in 4% jaggery broth for 10-14 days, harvest the mycelial mat and mix mycelial mat in soil @1% w/v while planting taro tubers.

Application/Beneficiary

P. indica could be utilized as PGPF in taro which leads to good yield.

Importance of the technology

P. indica was found to be efficient in promoting growth in taro varieties (Muktakeshi and Sree Kiran).

The PGP activity of *P. indica* could serve as a promising alternative to the PGPR available now.

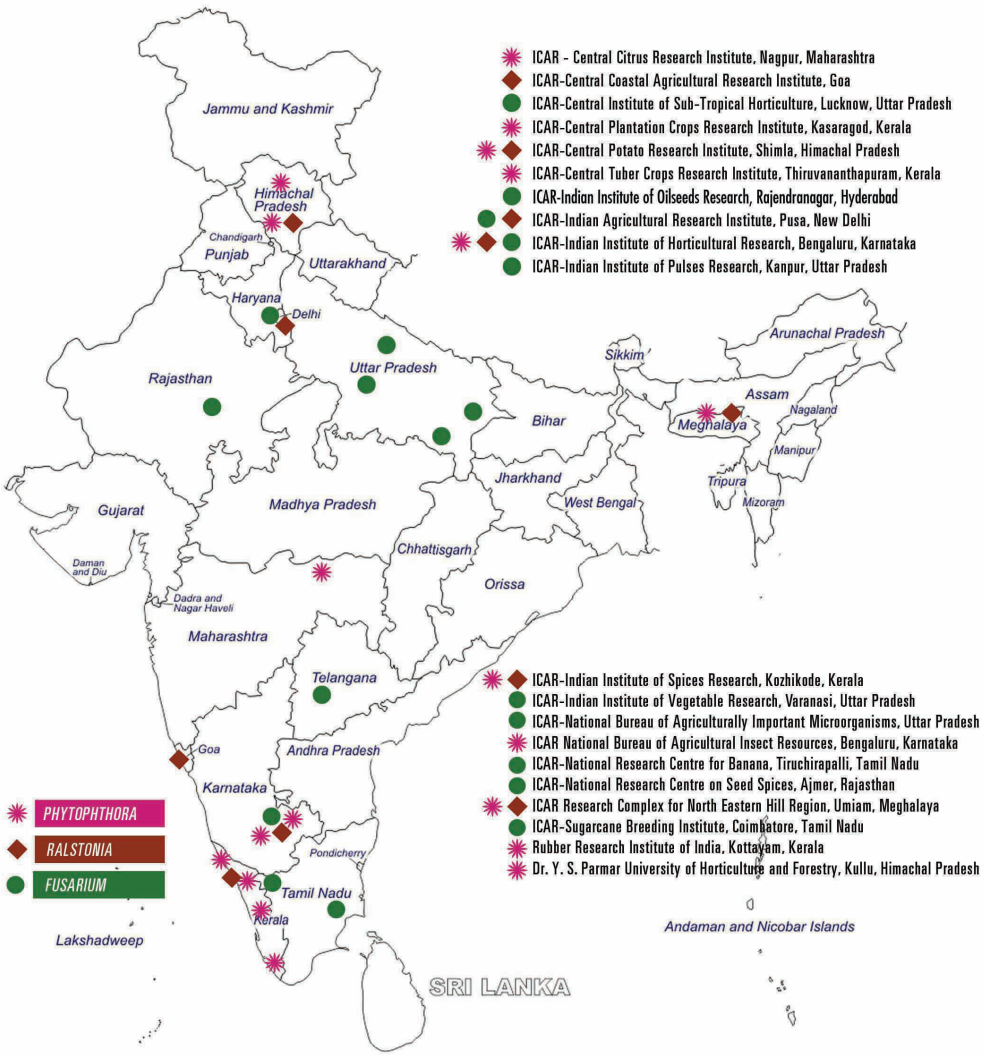
Status of commercialization

To be commercialized.

Keywords

Apoplastic bacteria 17
Apple collar rot 11
Bacterial wilt 14
Bacteriophage 21
Bacillus amyloliquefaciens 33
Bacillus flexus 29
Bacillus licheniformis 17
Bacillus subtilis 27
Bio-formulation 7
Banana 29
Black pepper 5
Brinjal 25
Calcium chloride 19
Citrus 7
Crossandra wilt 4
Cocoa stem canker disease 9
Coconut bud rot 9
Endophytic bacteria 23
Endophytic fungi 29
Fusarium wilt 21
Fusarium oxysporum f. sp. *gladioli* 16
Ginger bacterial wilt 19
Gladiolus 16
Grafting 15
Late blight 3
Penicillium pinophilum 29
Phytophthora cactorum 11
Phytophthora capsici 5
Phytophthora infestans 3
Phytophthora nicotianae 4
Phytophthora root rot 7
Potato 3
Piriformospora indica 34
Pseudomonas aeruginosa 1
Pseudomonas fluorescens 27
RNA interference 3
Radopholus similis 5
Ralstonia solanacearum 17
Rhamnolipid 1
Solanum torvum 26
Taro 34
Tomato 15
Trichoderma asperellum 13
Trichoderma harzianum 4
Xylem residing bacteria 24

LIST OF PHYTOFURA CENTRES



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