Green Approaches in Biotic Stress Management of Chilli Using Flourescent *Pseudomonas*

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Abstract

The present field investigation was carried out to study the effect of green approaches in Biotic Stress Management of Chilli Using Flourescent *Pseudomonas.* It has been found that the present trend is to practice integrated pest management by integrating various bio inputs such as fluorescent *Pseudomonas* with, pesticides, plant products such as neem cake, Farm Yard Manure and several other inputs to mitigate diseases and pests. Among the various plant growth promoting rhizobacteria (PGPR), fluorescent *Pseudomonas* is considered to be the most important since they have both plant growth promotion activity as well as production of anti-pathogenic secondary metabolites. Many of them promote plant growth by suppressing pathogenic microorganisms, synthesizing growth stimulating plant hormones, promoting increased plant disease resistance.The modern agriculture is highly dependent on chemicals for plant disease management. It is neither possible to suddenly switch over to biological control nor biocontrol alone will be able to replace chemical control. Hence, the present study aims at identification of potential fluorescent *Pseudomonas* isolates with broad spectrum antibiotics, such as 2,4, DAPG, phenazine, pyoluterine and other antimicrobial compounds, hydrogen cyanide, salicylic acid, siderophore, and PR proteins etc. The investigation also describes the compatibility of PGPR with pesticides, formulation, shelf life and its delivery as an integral component in sustainable management of biotic stresses in chilli.

Keywords: Green approach, biotic stress management, chilli, Flourescent, *Pseudomonas*

1. Introduction

Among the various plant growth promoting rhizobacteria (PGPR), fluorescent *Pseudomonas* is considered to be the most important since they have both plant growth promotion activity as well as production of anti-pathogenic secondary metabolites. Many of them promote plant growth by suppressing pathogenic microorganisms, synthesizing growth stimulating plant hormones, promoting increased plant disease resistance. The modern agriculture is highly dependent on chemicals for plant disease management. It is neither possible to suddenly switch over to biological control nor biocontrol alone will be able to replace chemical control. Therefore, their use as an integral component in the IPM is called for. Hence, the present study aims at identification of potential fluorescent *Pseudomonas* isolates with broad spectrum antibiotics, such as 2,4, DAPG, phenazine, pyoluterine and other antimicrobial compounds, hydrogen cyanide, salicylic acid, siderophore, and PR proteins etc. Further fluorescent Pseudomonas isolates were tested for their growth promotion in rice by inducing defence related enzymes such as peroxidise, polyphenol oxidase and phenyl alanine ammonia lyase. The investigation also describes the compatibility of PGPR with pesticides, formulation, shelf life and its delivery as an integral component in sustainable management of biotic stresses in chilli.

2. Material and Methods

2.1. Collection and characterization of fluorescent isolates of Pseudomonas

Isolates were collected from rhizophere of sorghum, wheat, chickpea, maize, safflower, sunflower, cotton, onion, pigeonpea and castor as well as from stem and root sections of the crops. The isolates were characterized and bacteriological tests were conducted as per Laboratory Guide for Identification of Plant Pathogenic Bacteria published by the American Phytopathological Society (Schaad, 1992). The antagonistic nature of isolates was carried out using dual culture test against plant pathogenic fungi.

2.2. Molecular detection of antibiotic genes

The detection of various antibiotic genes such as DAPG, phenazines, pyoluterine and pyrrolnitrin was carried out after isolation of genomic DNA from fluorescent *Pseudomonas*

isolates. The PCR amplification was run through. The various primers such as phl2a, phl2b, pltBf, pltBr, prnCf, prnCr, phzCD(f), phzCD(r), pvd(Af) and prd(Ab) were used for molecular detection of antibiotic genes such as 2,4, DAPG, phenazine, pyoluteorine, pyrrolnitrin and pyoverdine (Reshma et al., 2013, Naik et al., 2013).

2.3. Induction of systemic resistance

The susceptible rice variety, BPT 5204 was sued against sheath blight fungus *Rhizoctonia solani* for induction of systemic resistance by challenge inoculation. The seed treatment and root dippings were used for challenge inoculation. The assay of defence enzymes such as peroxidase, (PO) polyphenol (PPO) oxidase and phenyl alanine ammonia lyase (PAL) was done as per Reshma et al., (2013).

2.4. Shelf life and compatibility of bio-agent

Talc powder, vermicompost and farm yard manure (FYM) were used as carrier materials for mass multiplication of *P.fluorescens* (EP5) isolate. Suspension ($1X10^8$cfu ml^{-1}) of 400 ml of bacterial suspension was added to a kg of substrate. The inoculated substrates were mixed properly and sealed in polypropylene bag. The viable population of *P. fluorescene,* EP-5 isolate was determined at a monthly interval by serial dilution technique and expressed as cfu g^{-1} (colony forming unit) continuously for ten months.

2.5. Compatibility of P. fluorescens (EP-5) with fungicides, insecticides and plant products

The compatibility of EP-5 was assessed with fungicides, viz., Hexaconazole, Tricyclazole, Isoprothiolane and Propiconazole, and insecticides viz, Carbofuran, Imidaclorid, Fipronil, Buprofezin and plant products viz, neem seed kurnel extract (NSKE), nimbicidin, neem leaf and neem oil. The compatibility of PGPR bacterial isolate EP-5 was worked out with respect to *Trichoderma viride* using dual culture test.

2.6. Use of P. fluorescens in IPM

The present trend is to practice integrated pest management by integrating various bio inputs such as fluorescent *Pseudomonas* with, pesticides, plant products such as neem cake, Farm Yard Manure and several other inputs to mitigate diseases and pests. A large scale demonstration was conducted in farmer's field for three consecutive seasons. The IPM farmer beneficiaries were identified and provided with inputs and the regular interventions were made by monitoring pests and diseases throughout the season with judicious use of pesticides and bio-inputs. The observation on incidence of pests and diseases was recorded and the yield obtained was compared with that of without IPM practice.

3. Results and Discussion

3.1. Molecular detection of antibiotic genes

The large collection of fluorescent Pseudomonas isolates from the crop rhizosphere were tested for biocontrol efficacy against broad spectrum of pathogens such as *Fusarium solani Rhizoctonia solani, Macrophomina phaseolina Alternaria sesami Aspergilus flavus* etc. Molecular detection of 2, 4 DAPG genes by PCR analysis indicated the presence of DAPG in at least six isolates (Figure 1) with amplification of 745bp region which corresponds to DAPG gene. Veluswamy et al. (2006)

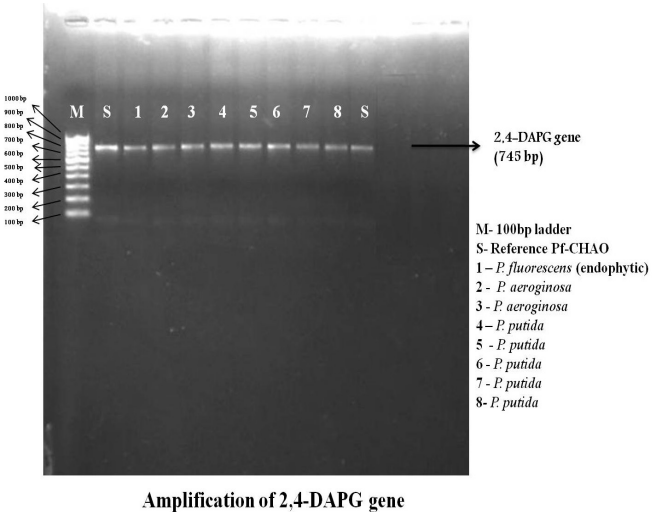

Figure 1: Molecular detection of 2, 4 DAPG genes by PCR analysis

also reported 27 strains of *P .fluorescens* showing DAPG out of 278 strains. The study revealed the occurrence of DAPG producers in Indian soils Manjunath et al. (2011) reported single strain of *P. putida* (CRFP-13) from brinjal rhizosphere possessing DAPG gene.

Phenazines, another antibiotic possessing broad spectrum antibiotic activity against plant pathogens. In the present study, three isolates of fluorescent *Pseudomonas,* EP-5, RP22 and RP46, showed the amplification of 1400 bp gene which corresponds to phenazine antibiotic gene. The same is in tune with observations of Zhang et al. (2006). Upadhyay et al. (2010) reported amplification of phZCD gene corresponding to phenazines. Such unique combination of antibiotics in any isolate may provide a competitive edge in the rhizosphere against plant pathogens.

Initial detection of siderophores was done by Chrome Azurol S agar (CAS). Two isolates (EP5 and RP46) produced siderophores on CAS medium but none of the isolates showed amplification of pvd gene with specific primers. None of the isolates showed the presence of plt gene by PCR assay with 779 bp known gene. The isolates were also found negative for amplification of Prn gene (Pyrrolnitrin). It is possible that the present isolates positive for 2,4-DAPG and phenazine may come under hydrogen cyanide producers based on the report of Nowak-Thompson (1999).

3.2. Induction of systemic resistance (ISR)

All the six fluorescent *Pseudomonas* isolates showed increased activity of defence related enzymes in rice when treated with fluorescent *Pseudomonas* isolates followed by challenge inoculation with *Rhizoctonia solani,* the casual agent of sheath blight.

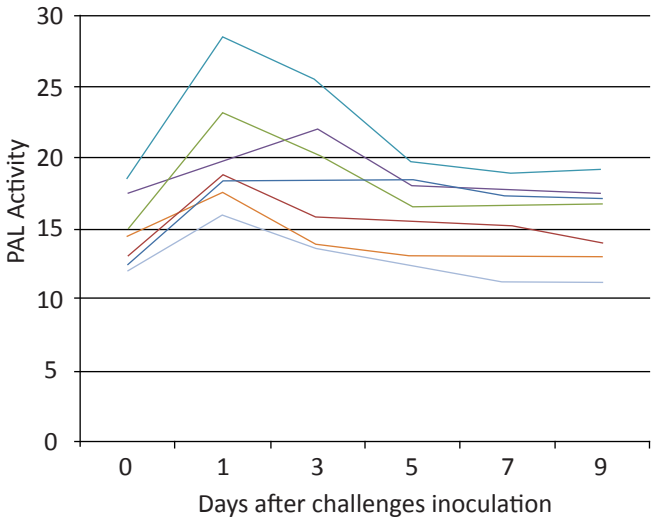

Figure 2: Induction of phenyl alanine ammonia lyase activity in Rice by seed treatment followed by root dipping with isolates of fluorescent Pseudomonas challenge inoculated with *R. solani* (R.s1)

Seed treatment followed by root dipping with fluorescent isolates initiated PO and PPO activity after 12 h of challenge inoculation with *R. solani* Maximum activity was recorded in treatment with EP-5 fluorescent isolate on the third day. However, maximum activity of PAL was recorded after 24 h challenge inoculation again with EP5 isolate (Figure 2).

followed by RP-22. The ISR mediated protection can reduce the disease severity as well increase the durability of ISR. The increased efficacy of combined application of biocontrol agents has been recorded in rice (Chatterjee et al., 1996; Vidyasekharan et al., 1997) and sugar cane (Vishwanathan and Samiyappan, 1999). Two peroxidase enzymes have been induced in PGPR treated rice plants inoculated with sheath blight pathogen (Nandakumar et al., 2001). Antibiotics contributing to ISR is reported by Kris et al. (2002) in tomato and Weller et al. (2011) in *Arabdiopsis thaliana* against *Pseudomonas syringae* in tomato.

3.3. Shelf life and compatibility of PGPR

Talc was the best carrier as it maintained uniform trend in mean population of *Pseudomonas fluorescens* (EP-5) over storage period of 10 months as compared to other carrier materials. The mean population was $19X10^7$ cfu g^{-1} for EP5 isolate (Figure 3).

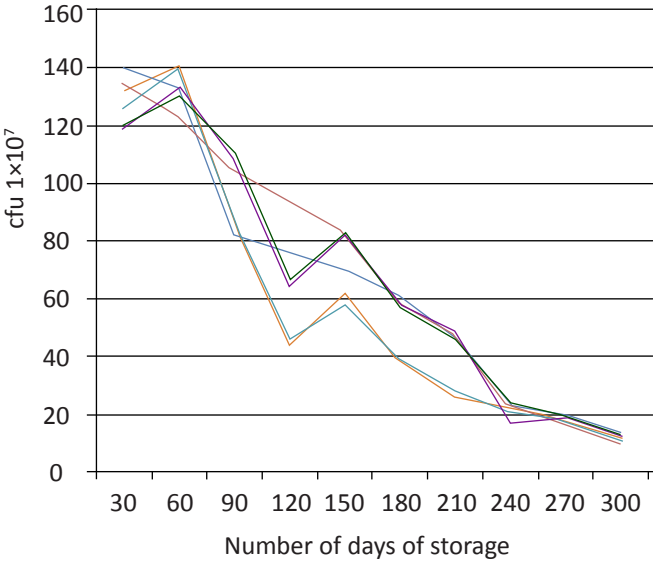

Figure 3: Effect of different carrier materials and storage temperature on population of EP5 isolate of *P. fluorescens*

The bacterium survived for five months in talc formulation. (Vidhyasekharan and Muthamilan, 1995). The results obtained by Sivakumar et al. (2000) and Jayaraman et al., (2007) indicated talc to possess best shelf life both at room and results are in tune with refrigerator temperaturesThe success of IPM depends upon how best various components of IPM are integrated and imposed. Hence, PGPR were tested for compatibility with fungicides, insecticides and plant products. The isolate EP5 was compatible with Hexaconozole, Tricyclazole and Propiconazole. Similarly, it was compatible with insecticides, Carbofuran and Imidachloprid. Jayakumar et al. (2003) noted the compatibility of *Pseudomonas* with Carboruran 3G and Avermectin. Kumar (2008) reported the compatibility of *P.fluorescens* with Imidachloprid and Carbofuran *P.fluorescens*(EP5) showed compatibility with NSKE, but not with neem oil (Figure 4).

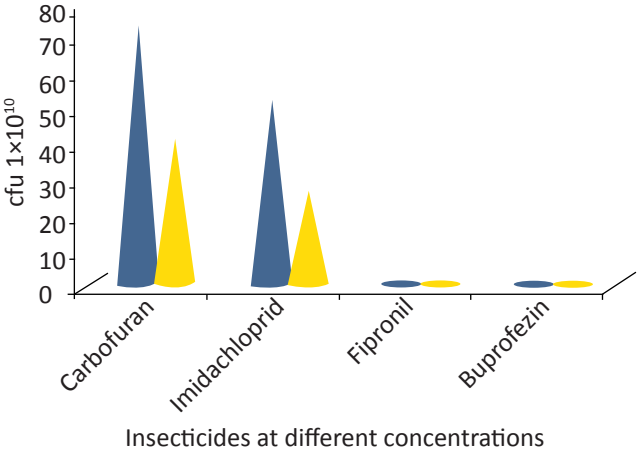

Figure 4: Compatibility of *P. fluorescens* (EP5) isolate with insecticide

The compatibility of *Pseudomonas* has been reported with carbendazim, thiram, Imidachloprid, Carbofuran and NSKE (Manjunath et al 2011).

3.4. Use of P. fluorescens as PGPR in IPM

The incidence of diseases and pests was monitored regularly in IPM and Non-IPM plots. The incidence of chilli leaf spot was in the range of 13 to 29%. The powdery mildew incidence ranged from 31-42%, root rot varied from 4-14% whereas fruit rot was in the range of 10-29% in the IPM plot. On the contrary, the incidence ranged from 15-32% for leaf spot, 33 to 45% for powdery mildew, 6-20% for root rot or wilt, 13-31% for fruit rot of chilli. The incidence of the disease came down and the number of sprays required for protection was significantly reduced by 30 % with increased yield and gross returns. The incidence of thrips infestation (1.92 leaf^{-1}), mites (3.3 leaf^{-1}), *Heliothis* (0.17 plant^{-1}) with1.36% fruit damage was observed in IPM plots as against thrips (3.26 leaf^{-1}), mites (5.2 leaf^{-1}), *Heliothis* (0.52 plant^{-1}) with fruit damage of 6.54% in non-IPM plot which reveals a significant difference. In addition, aflatoxin contamination was also at low level in IPM plot.

Introduction of *P. fluorescens* as seed treatment, soil application and foliar spray helped in achieving the goal of IPM for sustainable management of pests and diseases. Future of sustainable farming depends upon how best more of green pesticides integrated judiciously with that of other inputs not only to bring down the pest load but also for green and eco-friendly farming with care and concern for eco-system.

4. Conclusion

It can be concluded from the above that the incidence of chilli leaf spot was in the range of 13 to 29%. The powdery mildew incidence ranged from 31-42%, root rot varied from 4-14% whereas fruit rot was in the range of 10-29% in the IPM plot. On the contrary, the incidence ranged from 15-32% for leaf spot, 33 to 45% for powdery mildew, 6-20% for root rot or wilt, 13-31% for fruit rot of chilli. The incidence of the disease came down and the number of sprays required for protection was significantly reduced by 30 % with increased yield and gross returns.

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