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Exploring DAPG and Phenazine producing PGPR strains and fungal antagonists for the management of Noni diseases

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Summary and Conclusion

- Survey was conducted in different noni growing areas comprising of Tamil Nadu, Karnataka and Kerala states to assess the disease incidences. Highest incidence of leaf blight was noticed at Krishnagiri district, while lowest incidence was recorded in Madurai district of Tamil Nadu. In case of anthracnose, highest incidence was at Thrissur district of Kerala, while lowest incidence was recorded in Coimbatore district of Tamil Nadu. Theni, Madurai and Tuticorin districts of Tamil Nadu were also surveyed in which no incidence was recorded.
- The pathogens associated with the leaf blight and anthracnose diseases were isolated and identified through both morphological and molecular methods as *A. alternata* (Fr.) Keissler and *C. gloeosporioides* (Penz.) Sacc. Pathogenicity of both pathogens was proved under glass house conditions. Different methods of inoculation were studied. Among all, pin prick plus spore suspension spray was found to be best method. Virulence of the

isolates was studied, among fifteen isolates of *A. alternata* isolate AA1 was found to be highly virulent whereas, among ten isolates in case of *C. gloeosporioides*, C1 was found to be highly virulent.

- Morphological and molecular variation among the isolates of *A. alternata* and *C. gloeosporioides* were studied. The result revealed that there was a wide variation with respect to morphological and cultural characters of the isolates. With respect to molecular variation, among fifteen isolates of *A. alternata*, seven isolates from Tamil Nadu and two isolates from Karnataka formed one group. While the other three isolates belonging to Karnataka, two isolates from Kerala and one isolates from Tamil Nadu formed another group. In case of *C. gloeosporioides* out of ten isolates, five isolates from Tamil Nadu and one isolate from Kerala formed one group (Group A). Four isolates from Karnataka formed another group (Group B).
- Histopathological studies were carried out in leaves infected with *A. alternata* and *C. gloeosporioides* and the results indicated there was pink staining of cortex cells, and irregular thickening of cell wall with dense and granular cytoplasm in both case. In case of *C. gloeosporioides* infected leaves complete disintegration of palisade parenchyma was noticed.
- Different isolates of *P. fluorescens*, *B. subtilis* and *T. viride* were tested against both the pathogens under *in vitro*. Among all, Pf1 and TDK1 of *P. fluorescens* EPC8 and SVPR4 of *B. subtilis* and TV1 of *T. viride* were found to be highly effective against *A. alternata* and *C. gloeosporioides*, respectively. Compatibility among the effective bioagents was studied and all effective isolates were found to be compatible with each other.
- The induction of defense mechanisms in noni by different bioformulations treated plants showed increased activity of peroxidase, polyphenol oxidase, catalase and phenylalanine ammonia lyase in response to the leaf blight and anthracnose pathogens. The expression pattern of PO, PPO and proteins through native PAGE and SDS PAGE respectively, showed extra isoforms in bioformulations treated seedlings against pathogen inoculation.
- Efficacy of effective bioagents in vermicompost and neem cake based formulation as well as liquid formulations was tested against leaf blight

and anthracnose under glass house as well as field conditions. The result indicated that soil application of vermicompost, neem cake and Pf1+EPC8+TV1 mixture in the ratio 8:1:1 and foliar spray of liquid formulation of Pf1+EPC8+TV1 mixture @ 0.2% were highly effective in reducing incidence of leaf blight both under glass house and field conditions. In addition the same treatment recorded higher yield compared to other treatments. Similarly for anthracnose the treatment, soil application of vermicompost, neem cake and TDK1+SVPR4+TV1 mixture in the ratio 8:1:1 and foliar spray of liquid formulation of TDK1+SVPR4+TV1 mixture @ 0.2% was highly effective in reducing incidence besides increasing yield.

- The survey was undertaken to assess the incidence of fruit rot diseases in five districts across Tamil Nadu and Kerala. The result revealed that the highest incidence of dry fruit rot caused by *C. gloeosporioides* was observed to an extent of 71.40% in Coimbatore. The incidence of dry fruit rot caused by *A. alternata* was found to be maximum in Viasa of Trissur district (45.00%), The highest percent incidence of wet fruit rot was assessed in Coimbatore (63.3 %) it was followed by Aliyar Nagar (52.00 %) in Tamil Nadu.
- Noni fruit being affected by two types of fruit rot diseases namely dry fruit rot and wet fruit rot. Dry fruit rot is associated with *C. gloeosporioides* and *A. alternata* with marked difference in their symptoms, whereas soft fruit rot was caused by bacterial pathogen *P. agglomerans*. In pathogenicity test *C. gloeosporioides*, *A. alternata* and *P. agglomerans* reproduced fruit rot symptoms which were similar to natural infection.
- The causal organisms of dry fruit rot were identified as *C. gloeosporioides* and *A. alternata* based on their based on their morphological and cultural characters. The pathogen responsible for wet fruit rot was identified as *P. agglomerans* based on the morphological and biochemical characters further it was confirmed by FAME and 16s rDNA.
- Histopathological studies on dry fruit rot sample revealed structural changes and presence of pathogens propagules in infected fruit tissues. About eighteen *Bacillus* isolates consist endophytic and non endophytic were isolated from different crop growing areas and named BS1 to BS18.

Molecular detection of antibiotics biosynthetic genes revealed presence of surfactin gene in all 18 *Bacillus* isolates.

- *Bacillus* isolates BS1, BS2, BS3, BS4, BS6, BS7, BS8, BS10, BS11, BS13, BS14 and BS18 strains were amplified for iturin gene. Strains of *B.subtilis* BS1, BS2, and BS8 expressed bacillomycin gene. The genomic DNA of the strains BS1, BS2, BS4, BS6, BS7 BS8, BS11, BS14, and BS16and BS18 showed positive for fengycin gene.
- *In vitro* screening of *Bacillus* revealed the isolates BS2 showed highest inhibition against *C. gloeosporioides*, and *A. alternata* to an extend of 56.67 and 61.11 percent over control respectively. The antifungal activity of antagonistic *Bacillus* against *C. gloeosporioides*, and *A. alternata* was confirmed by observing abnormal swelling and lysis of conidia and mycelium of fungal pathogens scrapped from the zone of interaction.
- Antagonistic activity of *Bacillus* isolates against *P. agglomerans* revealed the isolate BS2 was found to record high inhibition zone of 28 mm. Crude antibiotics were extracted from *Bacillus* (BS2) using different organic solvents like ethyl acetate, methanol, hexane and butanol. Among different solvents used ethyl acetate extraction was found with high bioactivity against *C. gloeosporioides*, *A. alternata* and *P. agglomerans*.
- Ethyl acetate was used to extract crude antibiotics from BS1, BS2, BS6 and BS8 .Bioassay of crude antibiotics against *C. gloeosporioides* and *A .alternata* revealed isolates BS2 inhibited *C .goleosporioides* and *A. alternata* to an extent of 59.11% and 64.22 respectively. Antibacterial activity of crude antibiotics against *P. agglomerans* was tested among various isolate the BS2 recorded the maximum inhibition zone (29.00mm).
- Sensitivity of crude antibiotics from *Bacillus* spp to different temperature was assessed. The antimicrobial activity of crude antibiotics showed highest inhibitory activity against fruit rot pathogens at 40 ° C. But degree bioactivity was declined after exposure to 120 ° C for 15 min.
- Thin layer chromatography studies revealed that the *Bacillus* isolates BS1, BS2, and BS8 were observed the antibiotics surfactin and iturin with the R_f value of 0.3 and 0.7 respectively. GCMS studies on crude antibiotics

identified antimicrobial compound belonging to fatty acid, lipopeptide, peptide, and aldehydes which are responsible for antifungal and antibacterial activity.

- Finally the bio-efficacy of *Bacillus* isolates was tested under field condition. Among different dosages tested the foliar spray with 5 % of BS2 shown minimum disease incidence of dry and soft fruit rot.

-----End of statement-----