CHAPTER 6

SUMMARY AND CONCLUSION
Summary and Conclusion

The bacterial wilt disease caused by *R. solanacearum* (Smith) Yabuuchi *et al.* (1996) has long been a scourge of tropical, subtropical and warm temperate agriculture due to the wide distribution and unusually broad host range of the pathogen. Moreover, since *R. solanacearum* is a soil borne pathogen and host resistance is limited, bacterial wilt is very difficult to control.

As such, the present study aims to find a potentially ecofriendly measure for management of the bacterial wilt disease in the economically important and widely consumed horticultural crop, the brinjal (*Solanum melongena* L.) under local conditions.

Biological control is being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture and attention is diverted towards exploring the potential of beneficial microbes, for plant protection measures. Biocontrol is sustained by beneficial interactions resulting from competition, antagonism and hyper parasitism of certain microorganisms against plant pathogens, insects and weeds. Currently, several microorganisms involved in such processes are the active ingredients of a new generation of microbial pesticides. Biocontrol agents are easy to deliver, improve plant growth, activate resistance mechanism in the host, and increase biomass production and yield.

Therefore, the present study was undertaken to isolate an effective local strain of the established biocontrol agent *Pseudomonas fluorescens* and possibly find a green approach for controlling the plant pathogen.

The plant pathogen *R. solanacearum* was isolated from wilt infected brinjal plant collected from local brinjal plantations of Signimari in Kamrup district of
Assam, while the potential biocontrol agent, *P. fluorescens* was isolated from the rhizosphere rhizoplane of healthy brinjal plant growing in the local plantations.

Pathogenicity test was conducted for the isolated pathogenic strain. Morphological, physiological, cultural and biochemical studies were carried out for both the pathogen and biocontrol agent. Biovar determination was also carried out for the phytopathogen.

*In vitro* evaluation of antagonism of the isolated bacterial biocontrol agent, *P. fluorescens* against *R. solanacearum* was done and zone of inhibition was observed in dual culture assay. The minimum inhibitory concentration of *P. fluorescens* which could produce zone of inhibition in *R. solanacearum* plates was found to be $10^8$cfu/ml.

A pot experiment to examine the effectiveness of broth culture of *P. fluorescens* for the management of bacterial wilt disease was carried out in CRB design and PWI recorded. The antagonist suspension culture was applied by different methods before challenge with the pathogen for examining the effectiveness of the antagonist suspension in the management of bacterial wilt disease. The antagonist suspension @ $10^8$ cfu/ml applied as combined/ integration method of seed treatment, seedling dip and soil treatment proved best in reducing bacterial wilt incidence compared to other methods of application. The inoculated control treatment with only the pathogen inoculated and no prior application of the antagonist suspension showed 100% wilt incidence. Thus in the antagonist treatments, *P. fluorescens* could suppress the population build up of the pathogen and reduce the incidence of bacterial wilt as reflected from the PWI in such treatments. This is also supported from the population dynamics study of the pathogen and the bacterial biocontrol agent. The population of the pathogen successively decreased in the rhizosphere at the three crop stages.
sampled (30, 60 and 90 days after transplanting) while the bacterial biocontrol agent successfully established in the rhizosphere and multiplied to reach peak population at 60 DAT and only showed declining trend at 90 DAT.

A correlation study carried out for this experiment showed significant negative correlation between PWI and population of *P. fluorescens* and also between population of *P. fluorescens* and *R. solanacearum*. This further establishes the potential role of the biocontrol agent *P. fluorescens* to suppress the pathogen and reduce the incidence of bacterial wilt disease.

The effective biocontrol agent can be applied under field conditions or further commercialized only when immobilized in certain carriers. Thus formulations of the biocontrol agent should be prepared for easy application, storage, commercialization and field use.

With the above objective, different organic substrate carriers viz. Farmyard manure (F), Vermicompost (V), Decomposed mustard oil cake (D), Rice bran (Rb), Wheat bran (W), Rice straw (R) and Banana leaf (B) were evaluated for its effectiveness in the preparation of formulations. At the same time, three adhesives viz. carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA) and white flour gum (WFG) were also explored for their effectiveness in formulation development since adhesives in formulations help in adhering the biocontrol agent to the site of application in the plant. Mannitol was used as osmotica and CaCO₃ used for adjustment of pH during the preparation of the bioformulations.

The experiment resulted in establishing the formulation CVPf (carboxymethyl cellulose-Vermicompost – *P. fluorescens*) as the most efficient providing suitable nutrients and favourable niche as it supported highest population of the biocontrol
agent for a long period of storage. It was followed by CFPf, PVPf, PFPf, CDPf in increasing order of population recovery of the biocontrol agent.

Among the stickers, CMC proved to be the best followed by PVA and WFG supporting higher population of the biocontrol agent irrespective of the substrate carrier base. This could be attributed to the greater potential of CMC in supplying nutrients and protecting the microbes from desiccation and death.

For comparison of shelf life of the bioformulations when stored at different conditions, a set of the prepared bioformulations were also stored at 4°C. It was observed that even though the bacterial biocontrol agent *P. fluorescens* became metabolically inactive and could not multiply at 4°C, the rate of population decline at different days after storage sampled, was very slow and a comparatively higher population of the biocontrol agent was recovered at long 120 days after storage than the population recovered at room temperature. Thus after initial build up of population of the biocontrol agent in the substrate carrier adhesive based formulation at room temperature, subsequent storage at 4°C could be a suitable proposition for increased shelf life of the formulations.

Based on increased shelf life of the bioformulations five best formulations *viz.* CVPf, CFPf, PVPf, PFPf and CDPf were selected for applying by different methods in pot and field experiments for examining their effectiveness in controlling bacterial wilt disease.

The results followed similar trends in both pot and field experiments. The bioformulation CVPf applied by combined method of seed, root and soil proved most effective showing lowest PWI, highest population of biocontrol agent and lowest pathogen population in the rhizosphere of treated crops. Only uninoculated control
(no challenge with *R. solanacearum*) showed lower incidence of bacterial wilt. Among the bioformulations, CVPf was followed by CFPf, PVPf, PFPf and CDPf in decreasing order of effectiveness in controlling bacterial wilt. Among the methods of application, integration methods of application of the bioformulations proved more effective than single methods of application. Seed + root + soil method proved best while the seed method was the least effective in controlling bacterial wilt. The seed + root + soil method helped in inoculating higher dosage of the biocontrol agent which resulted in greater colonization of the rhizosphere by the biocontrol agent. Seed coating with the bioformulation increased the population load of antagonist on the seeds and protects the rhizosphere from the ingress of plant pathogens. Seedling dip or root treatment aids in the biocontrol agent coming in close proximity to the rhizosphere and proliferating there. Again, soil being the repertoire or both beneficial and pathogenic microbes, delivering of the antagonistic bacterial strain to soil increase the population dynamics of augmented bacterial antagonists and thereby would suppress the establishment of pathogenic microbes on to the infection court. Thus combining the advantage of each method, the seed + root + soil treated brinjal crops showed least incidence of bacterial wilt. On the other hand, the seed method proved least effective in the present study probably due to the small size of the brinjal seeds incorporating lower population dose of the antagonist and encountering of the treated crop with altered soil microenvironment during transplanting.

The study of the population dynamics of the pathogen and the biocontrol agent at 30, 60 and 90 DAT in both pot and field experiments showed that the pathogen population successively declined while the population of the biocontrol agent increased at 60 DAT and then showed declining trend at 90 DAT. The control treatments showed slow declining trend of the indigenous *R. solanacearum* and *P. fluorescens* populations present in the rhizosphere. In the treated crops, the increase in
population of the biocontrol agent reflects well establishment and proliferation of the biocontrol agent in the rhizosphere and rich nutrient base of the root exudates at the flowering stage (60 DAT). The decline at 90 DAT could possibly be due to the lower nutrient content of the root exudates with increase in age of the roots. The successive decrease in population of \textit{R. solanacearum} reflects the possibility of suppression of the phytopathogen by the biocontrol agent by various means viz. competition for nutrients and niche exclusion, antibiosis and host pathogen interaction (induced systemic resistance). The negative correlation between PWI and population of \textit{P. fluorescens} and between populations of \textit{P. fluorescens} and \textit{R. solanacearum} further established the potentiality of \textit{P. fluorescens} in the management of bacterial wilt disease.

The experiment conducted for studying the different physiological and biochemical parameters, yield and yield attributes of the different bioformulation treated crops showed that the yield and other parameters of the bioformulation treated crops were higher than the inoculated control (only pathogen inoculated). The ability of the different bioformulations to control bacterial wilt incidence followed the same trend of increase or decrease of yield and other parameters. CVPf followed by CFPf were most effective in controlling bacterial wilt incidence and also showed highest yield, fruit weight/plant, no of branches/ plant no: of fruits/plant, largest leaf area, longest, plant height, and highest content of chlorophyll, carbohydrate and protein. From the correlation study, it was found that there existed a highly significant negative correlation between PWI and yield, yield attributes and other physico-biochemical characters. The results of the present study clearly point to the plant growth promoting properties of the biocontrol agent \textit{P. fluorescens}. The direct effect of plant growth promotion by \textit{P. fluorescens} entails providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating uptake of certain plant.
nutrients from the environment. The indirect promotion of plant growth by *P. fluorescens* is by preventing the deleterious effects of phytopathogenic microorganisms. The ability of PGPR (*P. fluorescens*) to promote plant growth could be due to its ability to produce or change the concentration of plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene; antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics and cyanide; solubilization of mineral phosphates and other nutrients.

The experiments conducted in the present study for establishing the isolated strain of *P. fluorescens* as an effective biocontrol agent for management of bacterial wilt disease of brinjal caused by *R. solanacearum* and a plant growth promoting rhizobacteria increasing yield of brinjal crop and accelerating and improving the physico-biochemical properties, have however, been subjected to limitations. Large scale field trials under different climatic conditions prevailing locally are needed for further confirmation. Various environmental factors including climate, weather conditions soil characteristics or the composition or activity of the indigenous microbial flora of the soil that may affect the performance of the PGPR have to be taken into account. The indigenous soil microbial flora could also be explored for their interaction with the PGPR for finding possible beneficial effects of plant growth promotion that may be exerted by the PGPR in combination with these soil microflora.