SUMMARY

AND

CONCLUSIONS
Sunflowers are grown in many countries throughout the world and have been cultivated and harvested by many cultures for at least 4,500 years. During its long history, they have been used for several purposes that range from culinary to medicinal. One of the most important biotic constraints for sunflower (*Helianthus annuus* L.) production is the soil-borne obligate-parasitic oomycete *Plasmopara halstedii* (Farl.) Berl. & De Toni, which causes downy mildew disease of sunflower. The downy mildew pathogen attacks the plant at very young stage and spreads systemically throughout the life of the plant thereby resulting in yield loss. Sunflower downy mildew is presently managed by cultural methods, by use of chemicals and by the use of hybrid cultivars, and all these methods have their own limitations. Cultural methods are not always effective and the pathogen is variable and quickly breaks down resistance of hybrids. Furthermore, the use of harmful chemicals and fungicides results in residual effects, and also affects both environmental and human health. In this context, alternative approaches for management of sunflower downy mildew disease are being explored and one of such approaches gaining worldwide acceptance and importance is induction of resistance or plant immunization.

With this background knowledge in the present study, a total of 59 plant growth promoting fungi (PGPF) were isolated from the rhizosphere of different crop plants of different agro climatic regions of southern India. PGPF isolated were identified and evaluated for their effectiveness on growth promotion and induction of resistance in sunflower against the downy mildew disease. Screening experiments have revealed that out of 59 PGPF isolates, only seven fungal isolates were potential to promote growth and to induce resistance in sunflower. Enhanced seed germination and vigor index was noticed in PGPF treated seeds, when compared to the untreated control. Among the seven PGPF isolates, highest seed germination (92%) and vigor index (1871) was noticed in seeds treated with PGPFYCMTh (1×10^8 spores ml^-1), when compared to other treatments and the control. Application of these PGPF as seed treatment induces systemic resistance in treated plants and plants were protected against the incidence of downy mildew disease under greenhouse conditions. However, the ability of PGPF to initiate resistance in treated plants varied with the PGPF and type of treatment. The present study revealed that, the seeds treated with PGPFYCMTh (1×10^8 spores ml^-1) offered a maximum of 61% protection against the
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Disease followed by 58% in plants treated with PGPFYCM-Po of same concentration under the greenhouse conditions.

Similarly, in the second chapter along with PGPFYCMTh, other two strains of *T. harzianum* were further identified based on the molecular techniques and evaluated for their effectiveness to promote growth and induce resistance in sunflower against downy mildew disease under both greenhouse and field conditions. Treatment of sunflower seeds with conidial suspension and formulations of the three *T. harzianum* isolates produced desirable results in promoting growth and inducing resistance against downy mildew disease. However, the performance of the isolates in their ability to promote growth and induce resistance in sunflower varied considerably. Effectiveness of *T. harzianum* isolates as seed treatment, in promoting growth was evident from the initial stages itself, wherein seed germination, seedling vigor, seedling emergence and seedling stand were improved over the control under all tested conditions. The present study clearly demonstrated that under greenhouse and field conditions, seed treatment with both conidial suspension and formulations of *T. harzianum* PGPFYCM-14 and PGPFYCM-2 reduced the downy mildew disease incidence and offered a fair to good amount of resistance against the disease. The study also demonstrated the increased uptake of NPK nutrients in plants treated with *T. harzianum* isolates.

Further the best isolate *T. harzianum* PGPFYCM-14 which expressed a maximum protection against the disease under greenhouse and field conditions, was subjected to further investigations to study the biochemical mechanisms of induction of systemic resistance in treated plants. Experimental results have demonstrated the presence of higher levels of peroxidase, phenylalanine ammonia lyase, polyphenol oxidase, β-1,3 glucanase and lipoxygenase enzymes, in plants treated with *T. harzianum* PGPFYCM-14, which are said to have a role in plant defense mechanism. Time and magnitude of defense enzymes production in treated and resistant seedlings was more or less on far with each other. Observations of biochemical investigations are in line with the reports of triggering of defense system of plants, treated with biotic inducers such as PGPR and PGPF. The different temporal pattern of expression of these enzymes, suggests their differential role in induction of systemic resistance in sunflower against downy mildew disease. The very rapid and large changes in the resistant and *T. harzianum* PGPFYCM-14 treated seedlings, in contrast to the delayed
and smaller changes in the susceptible seedlings suggests that, the rate and magnitude of chemical defense responses are very important for the effective expression of defense mechanism against the invading pathogen. The activities of these enzymes act as markers of PGPF mediated induced systemic resistance in general and sunflower downy mildew interaction in particular. Additionally, subsequent studies were carried out in fourth chapter to investigate the mechanism of induction of resistance by *T. harzianum* PGPFYCM-14 against sunflower downy mildew disease at the molecular level by studying the pattern of accumulation of transcripts of some defense enzymes that are said to have a role in the systemic induction of resistance. Expression level of genes responsible for the production of defense enzymes like peroxidase, phenylalanine ammonia lyase and β-1,3 glucanase were studied by observing the temporal pattern of accumulation of their transcripts in treated seedlings and comparison was made with the resistant and susceptible seedlings. Accumulation of mRNA for each gene was measured in response to *T. harzianum* PGPFYCM-14 treatment and the same was compared with the resistant and susceptible seedlings with or without pathogen inoculation. The results have shown the presence of higher levels of transcripts in resistant and susceptible seedlings treated with *T. harzianum* PGPFYCM-14, in comparison with the untreated susceptible seedlings for the genes examined. *Trichoderma harzianum* PGPFYCM-14 treatment triggers transcript accumulation in treated plants when compared to the untreated control. Measurement of mRNA accumulation demonstrates that genes encoding *POX, PAL* and β-1,3 glucanase were triggered very prominently by *T. harzianum* PGPFYCM-14 treatment. Transcripts for each gene were also observed in susceptible seedlings, but not to the level of resistant and inducer treated seedlings. Finally, in the fifth chapter we have evaluated *T. harzianum* PGPFYCM-14 in the form of bioformulations for its biofertilizer and bio control efficacy. Under farmer’s field conditions, treatment of sunflower seeds with formulations of *T. harzianum* PGPFYCM-14 produced promising results by improving growth parameters and by increasing the crop yield when compared to the control. Similarly, significant reduction in downy mildew disease incidence was also noticed in the test rows raised with bioformulations treatment of *T. harzianum* PGPFYCM-14, when compared to the untreated control rows. On the whole, identification of an effective strain of PGPF- *T. harzianum* and testing its efficacy *in vitro* and *in vivo* conditions revealed
that it is an ideal and promising PGPF for sunflower downy mildew disease management in an eco-friendly manner. This study highlights the potentiality of *T. harzianum* PGPFYCM-14 in inducing systemic resistance in sunflower against the downy mildew pathogen, which can be used as an effective and safe alternative to chemical treatments for the control of sunflower downy mildew disease. The present study also throws light on the practical applicability of *T. harzianum* in sustainable agriculture for disease management and crop improvement. The future challenges for utilizing the identified *T. harzianum* PGPFYCM-14 lies in the successful development of effective formulations in large-scale and cost-effective production of the same. Thus more effective utilization of PGPF against a broad spectrum of plant pathogens, based on the inexpensive, renewable, eco-friendly and long lasting beneficial effects for host plants, will present major opportunities for plant researchers in days to come.