SUMMARY, CONCLUSION & RECOMMENDATIONS
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7.1 SUMMARY:

Nematode induced soil borne disease complex in floricultural crops like gerbera is seriously hampering the quality and quantity of yield of the flowers. This takes away heavy toll every year in the revenue to be obtained from exports of these exotic flowers. Exports of flowers are of one of the major sources for foreign exchange in the country.

This disease complex is caused by *M. incognita* and *P. cryptogea* in this crop. As the chemical control is expensive and hazardous, it was thought to use bio-agents for the management of this nematode induced disease complex in gerbera. Single bio-agent is not often effective in the management of disease complex caused by two pathogens (nematode and pathogenic fungus). Hence, after ascertaining the compatibility of *T. harzianum* and *P. fluorescens* it was thought to use these bio-agents for standardizing a strategy of the management of nematode induced complex in gerbera in protected as well as open field conditions.

In the present investigations, the major emphasis was on molecular characterization of *T. harzianum* for finding its nematicidal characters which helped in developing primers for molecular marker - PRA1 gene. This gene produces trypsin like protease which is a major nematicidal metabolite produced in *T. harzianum*. β-tubulin is the another gene on which the work was carried out which has both the nematicidal activity and also antifungal activity which will take care of nematode disease complexes in gerbera.
*T. harzianum* is a potential bio-control agent as they will affect the life cycle of nematodes in the rhizosphere.

To understand the interaction between *T. harzianum*, plant and nematode host, methods for *in situ* visualization of fungus are needed. This is possible by clubbing the approaches *viz*, isolation of the filamentous fungus of *T. harzianum* using TSM followed by its morphological, phenotypical, biochemical characterization which was again confirmed from ITS based detection of strains of *T. harzianum*. It was followed by detection of marker genes β-tubulin and PRA1 by PCR.

Successful isolation of different strains of *Trichoderma* sp. was done on TSM and at all the isolates 48 isolates having better colony characteristics, sporulation showing good growth rates was selected. They were all subjected to morphological, phenotypical, biochemical characterizations. Out of 48 strains, we selected 15 strains are *T. harzianum* for further studies. These were reconfirmed by molecular identification of species by ITS amplification. Then primers were developed for the marker genes PRA1 and β-tubulin as they represent the nematicidal activity.

Th-1, Th-2, Th-3, Th-4 and Th-5 are the 5 strains of *T. harzianum* were found to have β-tubulin and PRA1 genes.

These 5 strains were evaluated *in vitro* for quantification of their nematicidal activity from the 2 different concentrations (100% and 50%) of cell free culture filtrates obtained from *T. harzianum*. It was evaluated for its effect on hatchability of eggs and mortality of juveniles (J₂). The volatile nematicidal
compounds released as secondary metabolites in the broth during its growth are the direct products of genes PRA1 and β-tubulin.

These compounds are found to be effective against the hatching of eggs from the egg-masses and the suppression was higher in case of Th-3. It also has shown highest mortality of the freshly hatched juveniles of M. incognita. This was also confirmed through in vivo evaluation of these strains against root-knot nematodes on tomato.

Then another bio-control agent - P. fluorescens was used in combination of T. harzianum. Perusal of literature indicated that P. fluorescens increases the efficacy of T. harzianum. Then to go ahead using the combination of these two bio-agents, it was required to find the compatibility of IIHR-strains of T. harzianum with existing Pf1 and Pf2 strains of P. fluorescens in vitro. T. harzianum strain-Th3 was found to be highly compatible with Pf-1. Dry weight estimation experimental results also indicated that the same combination to be the highly compatible. Then the same combination was evaluated in vivo to find their compatibility and there also it has proved to be the best.

This combination was evaluated against P. cryptogea in vitro where it has proved its efficacy, showing up to 70% inhibition in dual culture methods. It has also shown inhibition when tested with single bio-agents, but the % inhibition was significantly more in case of combination of both bio-agents - Th-3 and Pf-1.
This combination was sorted out from others to test *in vivo* under screen house conditions to generate information on its bio-efficacy on the target nematode induced foot rot disease complex in gerbera. The experimental design was RBD (Randomized Block Design) with 10 different treatments. Treatment T9 (Th+Pf- SD+SB) with combination of both bio-agents @ 12.5 g each mixed with soil and seedlings given root dip treatment in the combination of these formulations had shown significantly higher plant growth parameters with least incidence of disease and nematode infection (Table 5.25).

CFU from 1g of root samples was taken and it was found that the extent of root colonization was significantly high in this treatment (Table No 5.18).

The egg mass parasitization was higher as the no. of J2 hatched from 10g of root sample was less and also the no. of eggs/ egg mass was also lesser compared to control and other treatments. It shows that the whole life cycle of *M. incognita* was affected by these bio-agents.

The % disease incidence was also lesser in case of foot rot disease as the entry of pathogens through the root galls was restricted due to reduction of nematode infection. There could also be a direct effect on this fungal pathogen due to vigorous colonization of these bio-agents in the rhizosphere which shows antagonism to the other fungal pathogens by restricting its growth. It was found from this experiment that the combination of formulations of *T. harzianum* and *P. fluorescens* was effective *in vivo* in gerbera under screen house conditions (Table No 5.23-5.25).
The data obtained from all the above experiments revealed valuable information on the management of nematode induced complex in Gerbera. We conclude that *T. harzianum* and *P. fluorescens* when used together help in effective management of nematode induced foot rot disease complex in gerbera.

Further we also evaluated this combination of formulations of bio-agents under poly-house and open field conditions by using organic substrates enriched with bio-agents for the effective management of the disease complex. So, two different cultivars of gerbera which suits poly house and open field conditions were selected for this evaluation.

Under field conditions, in the initial stages, bio-agents will have to fight for their survival first in the new environment after their introduction. Before they get colonized the root system, the pathogens should not suppress the growth of target crops. However farmers will not be able to spend huge amounts for the bio-pesticide application. Hence it was thought to use organic substrates *viz.*, neem cake, vermicompost and FYM which could be enriched by bio-agents by following standard protocols. This helped the survival of the organisms after introduction into the rhizosphere of gerbera in both protected and open field conditions.

Organic substrate enrichment with the bio-pesticides also helped in reducing the cost of application as the requirement of formulations of these bio-agents for enrichment was very less. Observation recorded on CFU of the organisms in the substrates after enrichment indicated that these bio-agents were multiplied in the substrates during the enrichment process.
The organic substrates, enriched by individual bio-agents and the combination of formulations of both bio-agents were used for the soil application. This enrichment method helped to get a multifold benefit as they enhanced the plant growth due to its organic nature amended with bio-agents.

Neem cake enriched with the bio-agents was significantly effective in decreasing the disease incidence (Table No.34). Neem cake also has the antifungal and nematicidal properties. Application of neem cake is proved to be very effective in the management of nematodes as it is reported to be controlling the entry of pathogens through variety of mechanisms including the production of antimicrobial compounds (Muller and Gooch 1982; Mankau, 1962; Alam et al., 1980). Thus it worked synergistically to antagonize the target pathogens along with *P. fluorescens* and *T. harzianum*.

*Pseudomonas fluorescens* being a Plant growth promoting rhizobacteria (PGPR) and both bio-agents show Induced Systemic Resistance (ISR) mechanisms, when applied to soil along with neem cake proved to be significantly effective in the management of a nematode induced disease complex in gerbera followed by a treatment of application of bio-agent enriched vermicompost.

These two substrates (neem cake + vermicompost) were combined after enrichment with both bio-agents and applied to soil @ 250 g. This treatment has significantly increased the yield to the highest when compared to other
treatments (Table-5.32 and 5.37) and also reduced the incidence of nematode and fungal pathogen (Table-5.33 and 5.37).

It was also found to have reduced the no of galls with a suppression effect on nematode hatching and also the fecundity of nematodes in the plant rhizosphere (Table- 5.32, 5.33 and 5.37).

In case of poly-house as the soil was pre sterilized the pathogens were inoculated to the threshold level to see the effect of treatments against the disease complex.

This bio-management strategy has helped not only in controlling the nematode induced disease complex but also enhanced the growth of plants resulting into increased yield of flowers up to 40% (Table-5.32 and 5.37).

The cut flowers obtained from these treatments beds also had a higher vase life which is an additional parameter for their quality which attracts the importers of these flowers abroad.

The bio-management strategy helps in the reduction of usage of hazardous chemical fungicides and nematicides. This also reduces the cost of production, can lead to golden revolution in the floriculture.

The strategies developed for gerbera crop can also be used as a model system for developing similar strategies in other crops for the management of similar disease complex in protected and also under open field conditions.
This strategy is a cost effective disease management systems using biopesticides and organic substrates which will not allow the development of resistance against the pathogens. This kind of comprehensive packages and methods of controlling diseases will also help in encouraging the farmers to grow crops by completely adopting organic methods of farming.

7.2 CONCLUSION AND RECOMMENDATIONS:

*Trichoderma harzianum* being widely accepted for its antagonistic nature against the fungal pathogens in crop plant was also known for its nematicidal activity. Thus it was thought to characterize the strains of *T. harzianum* for its nematicidal activity which will help in one step identification of the strains with bio-control activity.

Forty eight isolates of *Trichoderma* spp. from the rhizospheres of different agro-climatic regions of Karnataka, Tamil Nadu, Kerala, Andhra Pradesh and Himachal Pradesh were isolated. Out of which 15 strains were identified as *T. harzianum* by “Internal Transcribed Spacers” (ITS) region amplification by ITS1 and ITS4 primers.

Five out of the 15 *T. harzianum* strains responded positively for the detection of presence of both β-tubulin and PRA1 genes. This is the first report on identification for the presence of both nematicidal and antifungal genes in a single strain of *T. harzianum*.

The molecular marker primer sets developed for these two marker genes help in single step identification of the strains of *T. harzianum* for its antifungal and nematicidal activities.
The nematicidal activity of the cell free culture filtrates of these 5 strains at 2 different concentrations in vitro and in vivo evaluation proved that these 5 strains are efficient in controlling nematode infection by affecting life cycle of nematodes. **This is for the first time the cell free culture filtrates of *T. harzianum* was evaluated for the mortality in juveniles of *M. incognita*.**

Application of combination of *T. harzianum* with *P. fluorescens* to gerbera growing beds has proved to be more efficient in controlling nematode induced disease complex caused by *M. incognita* and *P. cryptogea* in gerbera. **This is the first report on the combination effect of *T. harzianum* and *P. fluorescens* in controlling this nematode induced disease complex on gerbera.**

The nematode induced disease complex caused by *M. incognita* and *P. cryptogea* is widespread causing severe loss in gerbera which is economically important floriculture crop in India in general and Karnataka in particular. Hence the present study was undertaken to develop bio-control strategies for the management of nematode induced disease complex in gerbera using a combination of formulations of bio-agents viz, *T. harzianum* and *P. fluorescens*.  

The efficacy of this combination was also proved in case of bio-management of nematode induced disease complex in protected as well as in open field conditions. The disease incidence has reduced up to 40% with an increase in the yield of flowers with prolonged vase life. **This is for the first time a complete organic method of bio-management was developed to control the nematode**
induced disease complex in gerbera in protected (polyhouse) as well as in open field conditions.

It is concluded that the investigations on “Molecular characterization of *Trichoderma harzianum* and Bio-management of disease complex in gerbera” were successfully completed by carrying out research work committed under the objectives.