DISCUSSION OF RESULTS
6. DISCUSSION OF RESULTS:

6.1 MOLECULAR CHARACTERIZATION OF SELECTED STRAINS OF T. HARZIANUM:

6.1.1.1: Survey for bio-agent strains and isolation of fungus *Trichoderma* spp.:

*Trichoderma* spp. was isolated from various agro-climatic regions of Southern India. It is very important to isolate indigenous strains from similar agro climatic regions so as to select efficient bio-control agents which would have been pre-acclimatized and can easily sustain the conditions of stress and high levels of antagonism from the local pathogens.

As we aim at bio-management of diseases of gerbera with the bio-control strains, containing genes which show high levels of bio-control activity against the pathogens, surveys were conducted keeping these in view and isolated various strains of *Trichoderma harzianum*.

6.1.2-3 Morphological and phenotypical characterization of *Trichoderma* spp.:

All the newly isolated strains were grown on different media to characterize morphologically on the basis of colony characters. *T. harzianum* was basically differentiated from different *Trichoderma* spp. on the basis of its alternating ring pattern on semi selective media, which is a light induced character (Rifai, 1969; Bisset, 1991).

Out of several strains, 48 strains were isolated and segregated for further experimental work based on their sporulation capacity, coloration and growth
rates. The strains with least capacity of spore production and growth rate were discarded.

They were further characterized phenotypically by microscopic observations as mentioned in the experimental methods for the ornamentation of conidiophores, spore structure and its size variations which differ from one species to another in the genus *Trichoderma*. This identification was not sufficient as many species have very minute variations or not much variations in the above characteristics. Hence carried out biochemical characterization which is supposed to give a better picture of differentiation.

**6.1.4 Biochemical characterization of *Trichoderma* spp.:**

Biochemical and physiological tests were carried out using readily available kits for the 48 strains which were newly isolated, as mentioned in the experimental methods. On the basis of the information available from the literature, 35 characters of reduction and utilization of certain carbohydrate sources and physiological tests based on change in pH were noted and documented in the experimental results section.

Each of the strain was characterized by its response to the biochemical and physiological tests. Their responses were rated as +++ followed by ++, + & - based on the intensity of response to each test.

These biochemical tests were not sufficient as they could not differentiate the closest related species. Their response to the biochemical and physiological tests were almost similar or un-differentiable.
The results obtained from the biochemical methods were confirmed by molecular methods of species differentiation.

6.1.5 Molecular identification of *Trichoderma* spp. by ITS regions:

Fifteen strains identified by biochemical methods as *T. harzianum* were again subjected to molecular detection of the species. ITS1 and ITS4 primers were used which amplifies the conserved regions of genomic DNA from ribosomal subunits. ITS1 and ITS4 are the forward and reverse universal primers used for the species level identification/differentiation (discussed in review of literature) in fungal organisms. These primers cover >90% of the region of internal transcribing region of genomic DNA.

The very minute variations in the nucleotide sequences can be identified by the PCR amplification of these primers. All the 15 strains were identified as *T. harzianum* even by molecular detection. Variations of 0.3 – 4.6 % were observed in all the sequences when BLASTed in NCBI. Even the one with highest variations also had shown 90% similarity with pre-available genomic sequences of *T. harzianum* submitted at NCBI. Thus we confirmed that all the 15 strains are *T. harzianum*. They were sub cultured, labeled and preserved for further work.

This step helps in elimination of any other species apart from *T. harzianum* from segregated collection of 15 strains for the next step of molecular characterization of *T. harzianum* for its nematicidal-bio-control property/activity.
It was required to design the primers for detecting the presence of target genes among the 15 strains. The primers were designed by Insilco methods from the available gene sequences of the target genes of fungal organisms collected from the databases. The similar gene sequences were collected online. They were all cumulatively edited by using the software program BIOEDIT. One nucleotide sequence of each of the target genes were obtained from this program. The obtained sequence was used to design the primers by using an online tool PRIMER 3. This tool has generated the primers which amplified maximum regions of the target gene. The primers for β-tubulin and PRA1 were designed. These primers annealed to the specific regions where the target gene is present and amplified only the target region hence indicating its presence.

The designed primers which were synthesized were subject for PCR program as mentioned in the experimental methods. The primers of β-tubulin have shown amplification in all the 15 strains of *T. harzianum*. Whereas, primers of PRA1 have shown amplification only in 5 strains of *T. harzianum*.

The results indicated that both β-tubulin and PRA1 were present in five strains. They were named as Th-1, Th-2, Th-3, Th-4 and Th-5. As β-tubulin in fungal organisms was known for antifungal and nematicidal activity and PRA1 was known for its specific nematicidal properties. Hence all these five strains were supposed to have high nematicidal as well as antifungal activities. As these 5 strains indicated the presence of targeted genes, they could be used as potential candidates for biological control of nematodes and other pathogens.
The primers designed and validated in the above experiments could be used as molecular markers for detecting the genes of PRA1 and β-tubulin in strains of *Trichoderma* spp. Thus we could predict these strains could be efficient strains with high nematicidal activity and also with antifungal activity.

So these 5 strains were tested *in vitro* and *in vivo* systems to validate their biocontrol activity against nematodes (*M. incognita*) and fungal (*P. cryptogea*) pathogens. Thus they were used for the further investigations for the bio-management of nematode induced disease complexes in gerbera.

**6.2 INVESTIGATIONS ON THE MODE OF ACTION OF *T. HARZIANUM* ON *M. INCognITA*:**

The results of molecular detection of the presence of the targeted genes *viz.*, PRA1 and β-tubulin for the nematicidal activity had to be evaluated *in vitro*. It is known that the organisms when grown in the liquid media release enzymes and secondary metabolites which are the end products of translation of the genes present in the organism. Thus, by conducting culture filtrate experiments we were able to validate the effect of the secondary metabolites present in the media released during metabolic activities of *T. harzianum*.

The mode of action of *T. harzianum* on the development and emergence of nematodes from the eggs in egg-masses was evaluated at 50% and 100% concentrations of culture filtrates. All the 5 strains of *T. harzianum* were tested for their efficacy to quantify the genetic expression of the genes PRA1 and β-tubulin. Out of 5 strains *T. harzianum* Th-3 strains was found to exhibit higher
nematicidal activity followed by Th-4, Th-1, Th-2 and Th-5 at both concentrations.

Another experiment was conducted in which the freshly hatched juveniles were subjected to two different concentrations of culture filtrates. Here high mortality was found in 100% concentration followed by 50% concentration of culture filtrates. In 100% concentration mortality of nematodes was observed from on 2\textsuperscript{nd} day only whereas in 50% concentration from 3\textsuperscript{rd} day onwards. The highest mortality was seen in Th-3 in 100% concentration followed by Th-4, Th-1, Th-2 and Th-5 at 100% concentration. The same pattern was seen in treatments with 50 \% concentration of culture filtrate also. Similar results were observed in the earlier experiments conducted in this report.

At higher concentration of culture filtrate, higher mortality of juveniles was observed.

The variation in suppression of hatching of nematodes and mortality rates in different strains could be due to slight variations in the genetic setup within the organisms or due to the occurrence of multiple copies of the nematicidal genes in the organism which results in the production of multiple folds of nematicidal compounds which would be relatively proportional to the no. of copies of the responsive gene.

These nematicidal compounds would be released into the media by the organisms during its growth, which are active even in the absence of the cell.
6.3 TO EVALUATE THE BIO-EFFICACY OF T. HARZIANUM AND P. FLUORESCENS AGAINST M. INCognITA AND P. CRYPTOGEA IN VITRo AND IN SCREEN HOUSE CONDITIONS.

6.3.1 Evaluation of compatibility of T. harzianum and P. fluorescens in vitro

Since T. harzianum alone would not able to manage both the targeted pathogens viz., M. incognita and P. cryptogea in the field conditions, in different soil types and in different agro-climatic conditions we used another compatible bio-control organism, for the management of nematode induced disease complex.

Trichoderma harzianum in combination with the same fungal species may not be best combination as both the strains in combination may work against the same pathogens / single pathogen. So, another biocontrol organism (P. fluorescens) having a broad host pathogenicity range was selected to evaluate its use in combination with T. harzianum for the bio-management of disease complex in gerbera.

To combine and use any two organisms it is necessary to check their compatibility in various conditions. Thus the compatibility of different strains of T. harzianum (Th1- Th5) with two available strains of P. fluorescens (Pf-1 and Pf-2) was evaluated to find the best compatible strains in vitro and in vivo (Table- 5.13).

To confirm the strains with a good compatibility, 4 sets of experiments were conducted by varying density of the presence of each organism in each of
the four methods. All the 4 methods were used to check the compatibility. The mean % compatibility was calculated from the average values of each treatment in all the experiments. The results indicated that combination of Th-3 strain of *T. harzianum* and Pf-1 strain of *P. fluorescens* were with high compatibility followed by Th-4, Th-1, Th-2 and Th-5 with Pf-1 respectively (Table – 5.13).

The results also indicated that growth rate of all the *T. harzianum* strains was enhanced when used in combination of *P. fluorescens* (Plates- 5.19 – 5.24).

6.3.2 Evaluation of compatibility of *T. harzianum* and *P. fluorescens* in vitro by mycelia dry weight method:

An experiment was then conducted to confirm the above results in which the dry weight of fungal mat was considered as a parameter to indicate the compatibility. In this experiment all five strains of *T. harzianum* were tested with Pf-1 strain of *P. fluorescens*.

The results of this experiment have confirmed the compatibility results obtained in earlier 4 experiments. The dry weight of Th-3 in combination with Pf-1 was highest, followed by Th-4, Th-1, Th-2 and Th-5 with Pf-1 respectively compared to that of the dry weight of the fungal mats of *T. harzianum* grown without inoculation of *P. fluorescens*.

With the data given in table 5.14 we observe that *P. fluorescens* enhancing the growth and growth rate of *T. harzianum* under *in vitro* conditions. These results were in correlation with the investigations of Siddiqui
and Shoukat, 2004, which says that metabolites produced by *P. fluorescens* enhance the growth of *T. harzianum* under *in vitro* conditions.

The CFU of these organisms in combination was also higher when compared to the CFU of these organisms when grown individually (Table – 5.15 and 5.16). This indicates that *T. harzianum* and *P. fluorescens* together seems to be having a synergistic effect on growth of each other in combination.

**6.3.3 Evaluation of compatibility of *T. harzianum* and *P. fluorescens* in *vivo*:**

The compatibility found *in vitro* methods were to be confirmed *in vivo* conditions too. All the five strains of *T. harzianum* were evaluated for its compatibility with Pf-1 and Pf-2 respectively *in vivo* with the treatments mentioned in the experimental methods.

Tomato was taken as a target crop for evaluation of compatibility of *T. harzianum* and *P. fluorescens* as it was possible to get precise data on this important aspect on tomato. The plant growth parameters recorded have shown significant increase in the growth of the plants treated with combination of formulations of *T. harzianum* and *P. fluorescens* (Table – 5.18). It was highest in case of treatments with both substrate and seed treatments with combination of both bio-agents.

The extent of colonization of roots with these bio-agents under *in vivo* conditions was recorded as CFU from 1g root sample. Results also indicated
*P. fluorescens* did not affect the colonization of *T. harzianum* on roots similarly. *T. harzianum* did not affect the root colonization by *P. fluorescens* rather there was increase in their colonization (Table – 5.17).

There seems to be synergistic effect of *T. harzianum* on *P. fluorescens* and vice versa in enhancing the plant growth along with the increase in substrate and root colonization. All these were possible as both the organisms were compatible under *in vivo* conditions. The results indicated that Th-3 in combination of Pf-1 was highly compatible followed by Th-4, Th-1, Th-2 and Th-5 with Pf-1 respectively.

Isolates of *T. harzianum* varying in their compatibility with *P. fluorescens* *in vitro* (Table 5.13 and 5.14) also varied in their efficacy *in vivo* as they increased root colonization and plant growth components (Table 5.16). The same variation pattern was observed under field conditions (Table 5.18). However, this variation was found proportional to the % compatibility between the strains (Tables 5.13- 5.18).

Hence, the results are useful to standardize the method of application of two or more bio-agents in combination for the effective management of disease complex of crops, as highlighted by different authors (Rao *et al.*, 1997; 1998a; Nagesh and Parvatha Reddy, 2000; Anusuya and Vadivelu, 2002; Manoj Kumar *et al.*, 2010).
6.3.5 Evaluation of bio-efficacy of various strains of \textit{T. harzianum} \textit{in vivo} against nematodes:

This experiment was conducted to evaluate the bio-efficacy of the \textit{T. harzianum} strains (Th-1 to Th-5) against nematodes under \textit{in vivo} conditions. Plant growth parameters and extent of colonization of root and colonization of egg masses was significantly higher in the strain Th-3 (Table – 5.22).

The strain Th-3 was showing highest efficacy against nematodes followed by Th-4, Th-1, Th-2 and Th-5 respectively (Table – 5.22). Similar results were also obtained from \textit{in vitro} bio-efficacy experiments were carried out using culture filtrates. We see clear cut correlation between \textit{in vitro} and \textit{in vivo} bio-efficacy experiments.

The data obtained from all the above experiments helped in selecting the best compatible strains of \textit{T. harzianum} and \textit{P. fluorescens} with relatively higher bio-efficacy. This initial straiinal selection helped us further to conduct investigations on the bio-management of disease complex in the target crop.

6.3.6 Evaluation of bio-efficacy of \textit{T. harzianum} and \textit{P. fluorescens} against nematode induced disease complex in gerbera under screen house conditions:

This experiment was conducted to evaluate the selected combination of strains of \textit{T. harzianum} and \textit{P. fluorescens} for their efficacy in management of disease complex under screen house conditions. This model experiment yielded
valuable information regarding the bio-efficacy of the combination formulation on nematode induced disease complex caused by *M. incognita* and *P. cryptogea* in gerbera.

This information also helped in development of strategies for efficient management of this nematode induced disease complex under practical conditions of poly-house and field in gerbera. In this experiment, the treatments with combination of *T. harzianum* and *P. fluorescens* applied for both seedling and substrate, proved to be significantly effective in the management of disease complex caused by both *M. incognita* and *P. cryptogea* (table 5.23).

The observations recorded were extent of root colonization of both bio-agents (CFU), plant growth parameters, root galling index of *M. incognita*, % suppression of nematode hatching from the egg masses collected from the infected roots colonized with both the bio-agents and no. of nematodes in 10g of root sample and disease incidence of foot rot caused by *P. cryptogea*.

Analysis of the results indicated that, Th3+Pf1 combination to be significantly effective when applied for seedling and substrate in gerbera under screen house conditions. There was a significant reduction in the no. of galls/cm of roots, no of nematodes in 10g of root sample and no. of eggs in an egg mass in this treatment (Table 5.24).

The reduction in no. of galls could be due to expressed nematicidal activity by *T. harzianum*. The reduction in the no. of eggs in the egg-mass
indicated Th3 + PF bioagents affected the reproduction capacity of *M. incognita* (Table 5.25).

Nematodes are known to usually assist and enhance the pathogenicity mechanism of the fungus though the modifications in tissue physiology of host plants (Shukla and Swarup, 1970; Chhabra and Sharma, 1981). Nematodes interact with different groups of plant pathogens and root symbionts. It seems reasonable to expect that infection by one pathogen may alter the host response to subsequent infection by another (Taylor, 1990). The increased lateral root production following nematode invasion also provides entry sites for fungi (Booth and Stover, 1974). The direct entry of *P. cryptogea* was reduced due to reduced nematode population leading the reduction in the % disease incidence also (Table 5.25). Disease incidence by *P. cryptogea* was found reduced as the nematode population in the root decreased (Table 5.25).

There were significant increases in the shoot length, shoot weight and root length, root weight in T9 (Table 5.25). Interaction involving nematodes and pathogenic fungus contribute substantially to variability in crop growth (Zadoks and Schein, 1979). Hence, the increase in plant growth (plant length and weight) in our experiments, could be also due to reduction in nematode infection and % disease complex. There was also significant increase in the yield in treatments with combination of *T. harzianum* and *P. fluorescens* (Table 5.25).

Siddiqui and Shaukat (2004) reported that the rhizosphere colonization pattern of two *P. fluorescens* strains, used individually or in combination with
*T. harzianum*, did not differ significantly, while it did differ in our experiments (Table 5.25).

When compared with treatments of individual bio-agents of different strains, the treatments with a combination of bio-agents were also reported to give better performance in previous studies (Rao *et al.*, 2004; Rao, 2007, 2008).

However, in some reports the feasibility of combining *Trichoderma* spp. with fluorescent pseudomonads was questioned (Hubard *et al.*, 1983). According to these authors, indigenous populations of fluorescent pseudomonads significantly reduced the biocontrol activity of *Trichoderma* spp. applied to control *Pythium* seed rot of pea and iron competition was the primary mechanism involved. In contrast, Dandurand and Knudsen (1993) reported that the combination of *P. fluorescens* 2-79 and *T. harzianum* ThzID1 neither inhibited nor enhanced the biocontrol activity of the latter agent against root rot of pea caused by *Aphanomyces euteiches* f. sp. *pisi*. Instead, our findings have shown a reduction in the disease complex in the plants along with increase in the yield (Table 5.25) when *P. fluorescens* and *T. harzianum* were used together compared to single bio-agent treatments.

It was also observed that mortality rate was higher in the J2 collected in distilled water from the roots and egg-masses. This effect could be due to effect of nematicidal compounds of bio-agents on eggs in egg-masses.

The % disease incidence caused by *P. cryptogea* was also found to be reduced. This could be due to direct effect of bio-agents by their cell wall
degrading enzymes on *P. cryptogea* and also because of restriction of the entry of pathogen into roots.

This clearly demonstrates that the nematodes and their entry into root system cause injury to the roots which is further colonized by *P. cryptogea*. There was reduction in the number of sites of nematode entry were less on roots decreased the entry of *P. cryptogea* leading to the lesser incidence of disease. Thus reduction in the galls and nematode infection has in turn reduced the secondary infection caused by *Phythophthora cryptogea* This in turn reduced the nematode induced disease complex in gerbera.

Thus the use of combination of *T. harzianum* and *P. fluorescens* has proved to be efficient in management of the disease complex in gerbera.

### 6.4 DEVELOPMENT OF A STRATEGY OF BIO-MANAGEMENT OF *M. INCognITA* AND *P. CRYPTOGEA* ON GERBERA IN POLY-HOUSE CONDITIONS USING *T. HArZIANUM* AND *P. FLUORESCENS*.

A set of four experiments (A, B, C & D) were designed for developing strategies for the bio-management of nematode induced disease complex caused by *M. incognita* and *P. cryptogea* under protected (polyhouse) conditions. Initially 3 experiments (A, B and C) were carried out with few common treatments useful for comparing the results.

Each experiment was designed with the treatments containing various combinations of formulations of both the bio-agents enriched in different substrates *viz.*, neem cake, vermicompost and FYM in set A, B and C respectively.
These organic substrates help the bio-agents to multiply faster in the rhizosphere and further aids in vigorous colonization on the root system. Being organic in nature these substrates would affect the beneficial soil microflora and also helps in plant growth.

It was inferred from the experimental results that the use of combination formulation of *T. harzianum* and *P. fluorescens* enriched in neem cake for application to seedling and substrate, to be the best treatment. This treatments has significantly reduced the nematode and disease incidence in Gerbera when compared with other treatments where in *T. harzianum* and *P. fluorescens* were enriched in vermicompost / FYM and used in the experiments. There was also significant increase in the yield (spike no.) of flowers where ever neem cake enriched with TH+PF was used (Table 5.33).

It was also observed that the combination of formulations containing *T. harzianum* and *P. fluorescens* enriched in vermicompost and their application to seedling and substrate was found to be at par with best treatment in case of yield.

These results indicate that the neem cake having the antifungal property helped in combating the disease complex along with the bio-agents synergistically.

In case of vermicompost, we observe that it has given a strong base for colonization of *T. harzianum* and *P. fluorescens* on gerbera. Nutrient contents of vermicompost helped in the production of more roots meeting the needs of the bio-agents to grow and multiply in the initial stages of application. To be
precise, vermicompost role helped in vigorous development of the root system triggered higher colonization of bio-agents on roots.

In set D it was thought to combine these substrates along with the application of both bio-agent formulations to control the disease complex in gerbera. Through this experiment it was thought to see the cumulative effect of neem cake + vermicompost along with the enrichment of combination of *T. harzianum* and *P. fluorescens.*

The results indicated that there was a significant reduction in the incidence of disease complex and also an increase in the yield of no. of spikes.

The substrate treatment with neem cake + vermicompost which were enriched with combination of *T. harzianum* and *P. fluorescens* and subsequent applications at an interval of 90 days proved to be the viable strategy for the management of disease for the sustainable increased in the yield of gerbera.

*Pseudomonas fluorescens* (Migula) belongs to PGPR, the important group of bacteria, which play a major role in the plant growth promotion, induced systemic resistance, biological control of pathogens etc. PGPR are known to enhance plant growth promotion and reduce severity of many fungal and nematode diseases (Haas, 2005; Manoj and Rao, 2011a). *Pseudomonas fluorescens* has capabilities of induction of growth promoting substances being a PGPR and induction of systemic resistance against pathogenic fungi (Ramamoorthy *et al.*, 2001).

The reduction in disease incidence by *P. cryptogea* could be due to antagonistic capabilities of *T. harzianum* against phytopathogenic fungi by
means of faster substrate colonization as competition for nutrients, antibiosis, and mycoparasitism by chitinolytic and glucanolytic enzymes as described by (Yedidia et al., 2003; Benitez et al., 2004; Reithner et al., 2011). In addition to the nematicidal mechanisms of *T. harzianum* described by Saurez et al. (2004), use of *P. fluorescens* which has ability to control nematode population in soil by siderophore production could have reduced the nematode population much significantly. It was also reported that certain strains of *T. harzianum* enhance the production of nematicidal compounds *in vitro* and improve biocontrol of nematodes by *P. fluorescens* (Siddiqui and Shaukat, 2004). Thus the nematode infestation has decreased significantly by use of the combination of both bioagents (Table 5.27, 5.29, 5.31 and 5.33). *P. fluorescens* has been reported effective in the management of root-knot nematodes (Parveen et al., 1998) and fungal pathogens (Xu and Gross, 1986; Cronin et al., 1997).

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 (Alam and Khan, 1980; Muller and Gooch, 1982). Vermicompost was found to be most suitable organic material which could be enriched with the bio-agents for the application in the main field conditions as it can help in the overall plant growth (Lazcano et al., 2009) simultaneously enhancing the microbial growth in the rhizosphere (Norman and Edwards, 2005). Effect of vermin compost containing humic substances at low concentrations has been explained by various theories, the most convincing of
which hypothesizes a “direct” action on the plants, which is hormonal in nature, together with an "indirect action" on the metabolism of soil microorganisms (Norman and Edwards, 2005).

Combined application of *T. harzianum* and *P. fluorescens* with neem cake and vermicompost also did not affect their colonization on roots of gerbera (Table 5.33). Rather the mixture of substrates helped in the increased colonization of both bio-agents (Table 5.33). Root colonization of *T. harzianum* and *P. fluorescens* was more when applied together with neem cake and vermicompost in comparison to individual treatments (Table 5.33).

Nematodes are known to usually assist and enhance the pathogenicity mechanism of the fungus though the modifications in tissue physiology of host plants (Shukla and Swarup, 1970; Chhabra and Sharma, 1981). Nematodes interact with different groups of plant pathogens and root symbionts. It seems reasonable to expect that infection by one pathogen may alter the host response to subsequent infection by another (Taylor, 1990). The increased lateral root production following nematode invasion also provides entry sites for fungi (Booth and Stover, 1974). The direct entry of *P. cryptogea* was reduced due to reduced nematode population leading the reduction in the % disease incidence also (Table 5.33). Disease incidence by *P. cryptogea* was found reduced as the nematode population in the root decreased (Table 5.33).

There were significant increases in the shoot length, shoot weight and root length, root weight in treatment T9 (Table 5.32). Interaction involving
nematodes and pathogenic fungus contribute substantially to variability in crop growth (Zadoks and Schein, 1979).

Thus bio-management of nematode induced foot rot disease complex caused by *P. cryptogea* and *M. incognita* was achieved by these strategies of combination of bio-agents of *T. harzianum* and *P. fluorescens*. This has also proved to be cost effective strategy as the bio-agents were enriched in the organic substrates earlier to the treatment, increased the propagule load of bio-agents. In this manner for enriching these organic substrates we require very small quantities of bio-agents were used for enrichment. The cost benefit ratio was calculated as 1:3.8 in this case.

### 6.5 STANDARDIZING THE METHODS FOR BIO-MANAGEMENT OF DISEASE COMPLEX IN OPEN FIELD CONDITIONS.

The treatments in experiments designed were same as that of four experimental treatments set -A, B, C & D used for the bio-management in poly-house and executed in the same manner with a change in dosages (mentioned in experimental methods in section 4.5).

The results were similar with that of the results of poly-house. These experiments were conducted in the sick plots in open field conditions. As these pathogens were present in the rhizosphere, in this case inoculation with pathogens was not done. Incidence of nematodes and *P. cryptogea* from 5 replicates were randomly evaluated and found that the treatment - TH+PF+NC+VER –SB had the least disease incidence with highest yield (Table-5.37). This was due to substrates enrichment with *T. harzianum* and
*P. fluorescens* which helped in the sustainable disease management with increase in yield of flowers.

Thus bio-management of nematode induced foot-rot disease complex in gerbera was achieved with repeated applications of neem and vermicompost enriched with combination of *T. harzianum* and *P. fluorescens* at regular intervals (Table- 5.37).