EXPERIMENTAL RESULTS
5. Experimental Results:

5.1 Molecular characterization of strains of *P. chlamydosporia*

5.1.1 Bio-agent isolation

5.1.2 Isolation of bio-control agent *P. chlamydosporia* through surveys

With the objective of isolation of indigenous strains of *P. chlamydosporia* surveys were undertaken in various regions such as Kolar, Tumkur, Dharwad, Doddaballapur, Shimoga (Karnataka) and Nelliampatti (Tamil Nadu) to collect root and soil samples from the fields of tomato, capsicum and okra affected with root-knot nematodes and nematode induced disease complex (Table no. 5.1). Keeping in view the isolation of native bio-agents, roots of tomato, capsicum and okra were selected for isolation of *P. chlamydosporia*. Fungus *P. chlamydosporia* was isolated as per methods given by Kerry *et al.*, (1993) on semi selective medium. Plate no.1 indicates the white colored colonies of the bio-agent *P. chlamydosporia* isolated on semi selective medium.
Plate No. 1 Isolation of bio-agents on *Pochonia chlamydospora* Specific Media

Colonies of *Pochonia chlamydospora* isolated on semi selective media.
5.1.2 Characterization of bio-agent based on Morphology:

*P. chlamydosporia* was successfully isolated using semi selective medium. Four different strains were isolated and numbered as PC-1, PC-2, PC-3, PC-4. The colony morphology of the cultures can be seen in plate no.2 which indicates the growth of 2 week old cultures on PDA plate as viewed from above and plate no.3 indicates the growth of 2 week old cultures on PDA plate as viewed from below. Plate no. 4 indicates the sporulation pattern of the four different cultures PC-1, PC-2, PC-3, PC-4 isolated.
Plate No.2 The growth of *P. chlamydosporia* on PDA (2 week old culture) - I

Colony colour of four isolates of *P. chlamydosporia* as viewed from above
Plate No.3  The growth of *P. chlamydosporia* on PDA (2 week old culture) - II

Colony colour of four isolates of *P. chlamydosporia* as viewed from below
Plate No. 4 Colony Morphology of Different strains of *P.chlamydosporia*
Plate No.5: PHENOTYPIC CHARACTERIZATION OF BIOAGENTS

Fungal Structures indicating the verticillate natures of the four isolates
5.1.3 Characterization of bio-agent based on Phenotype:

Colonies of *P. chlamydosporia* formed on semi selective medium were further sub cultured on two different media Potato Dextrose Agar (PDA) and Corn meal Agar (CMA). The plates were observed for the presence of verticillate nature of mycelium. The colony morphology was tested under microscope at 10, 20 and 40x magnifications. The verticilicate nature of the fungus was seen. Four different strains were isolated and numbered PC-3, PC-2, PC-1 and PC-4. The growth patterns of the strains were seen in these two media. Plate no. 5 indicates the presence of verticillate nature of four different cultures PC-1, PC-2, PC-3, PC-4.

5.1.4 Confirmation of *P. chlamydosporia* based on chlamydospore production.

The chlamydospore was produced as per the protocol described. Plate no. 6 shows the production of chlamydospores on the sand and barely media. Good number of chlamydospores was obtained from the two isolates of *P. chlamydosporia*. Chlamydospore production by the different isolates of *P. chlamydosporia* confirmed that the isolated filamentous fungus was *P. chlamydosporia*. Results on chlamydospore production revealed that there is significant difference between the strains PC-3 and PC-2 (Table No.5.2). Isolate PC-3 produced significantly higher number of chlamydospores $13 \times 10^8$ followed by PC-2: $8 \times 10^8$ followed by PC-1: $7.2 \times 10^6$ and PC-4: $4.2 \times 10^4$. 
Plate No.6 Chlamydospore Production on Sand and Barley

Plate No.7 Chlamydospore Production Media by *Pochonia chlamydosporia* (PC-3 isolate)
Table No. 5.1 List of the regions and source of Collection of Bio-gents.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Strain no.</th>
<th>Place</th>
<th>Source</th>
<th>Bio-agent Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PC-3</td>
<td>Kolar-Karnataka</td>
<td>Root</td>
<td><em>P. chlamydospora</em></td>
</tr>
<tr>
<td>2</td>
<td>PC-2</td>
<td>Tamil Nadu, Neelayampatti</td>
<td>Soil</td>
<td><em>P. chlamydospora</em></td>
</tr>
<tr>
<td>3</td>
<td>PC-1</td>
<td>Tumkur-Karnataka</td>
<td>Soil</td>
<td><em>P.chlamydospora</em></td>
</tr>
<tr>
<td>4</td>
<td>PC-4</td>
<td>Dharwad - Karnataka</td>
<td>Soil</td>
<td><em>P. chlamydospora</em></td>
</tr>
</tbody>
</table>

Table No. 5.2 Studies on variations in production of chlamydospores and root colonization levels of *P. chlamydospora* against *M. incognita* infecting Okra.

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Root colonization (CFU/g root)</th>
<th>Number of chlamydospores formed per gram of substrate</th>
<th>In vitro egg parasitization (%)</th>
<th>Rating of bio-efficacy of bio agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-3</td>
<td>8436</td>
<td>13x10^8</td>
<td>87.11</td>
<td>Very efficient</td>
</tr>
<tr>
<td>PC-2</td>
<td>7735</td>
<td>8x10^8</td>
<td>75.32</td>
<td>Efficient</td>
</tr>
<tr>
<td>PC-1</td>
<td>2347</td>
<td>7.2 x10^6</td>
<td>57.84</td>
<td>Inefficient</td>
</tr>
<tr>
<td>PC-4</td>
<td>2125</td>
<td>4.2 x10^4</td>
<td>52.26</td>
<td>Inefficient</td>
</tr>
</tbody>
</table>
5.1.5 Identification and conformation of *P. chlamydosporia* through Molecular studies:

The purified PCR product of *P. chlamydosporia* internal transcribed regions which were amplified using the universal primers ITS4 and ITS5 (Table No.5.3) were sent for sequencing to Bioserve, Hyderabad. The sequences of the ITS regions were compared using the blast tool in NCBI server against the already existing fungal database there was similarity of our culture PC-3 and PC-2 culture with *P. chlamydosporia*. Thus the identity of the isolated strains (PC-3 and PC-2) was confirmed as *P. chlamydosporia* through ITS region amplification. Fig No.1 indicates the 99% similarity of our culture PC-3 with *P. chlamydosporia* and Fig No.2 indicates the 97% similarity of PC-2 culture with *P. chlamydosporia*. 
Fig No.1 Blast results indicating the species level of identification of *P. chlamydosporia* (PC-3) using ITS4 and ITS5 primers.
Fig No.2 Blast results indicating the species level of identification of *P. chlamydosporia* (PC-2) using ITS4 and ITS5 primers
5.1.6 DNA isolation from the strains of *P. chlamydospora*:

The genomic DNA was successfully isolated using the Rader and Broder method. DNA isolation from 2g of one week old culture of *P.chlamydoospora* yielded good quality of high molecular weight DNA and was visualized by agarose gel electrophoresis. The optimum concentration of DNA to be taken for polymerase chain reaction was standardized by running on agarose gel (1%). DNA concentration that gave very bright band under UV light was taken for the further studies.

5.1.7 Amplification of β-tubulin gene by polymerase chain reaction using tub1f and tub1r primers.

Polymerase chain reaction was carried out to detect β-tubulin gene using the specifically designed primers. The gel picture analysis revealed that the polymerase chain reaction product obtained was 270bp, which clearly indicated the detection of β-tubulin gene.

Plate No.8 Gel picture showing β-tubulin amplification at 270bp

Plate No.8 indicates the gel picture showing the β-tubulin gene amplification at 270bp.
Fig no.3 Blast results indicating the presence of beta tubulin gene in PC-3 strain.
Fig No. 4: Blast results indicating the presence of beta tubulin gene in PC-2 strain.
The amplified Polymerase chain reaction product was sent for sequencing. The sequencing results were then compared using the BLAST tool against the available sequences at NCBI. The blast result indicated that this sequence was β-tubulin gene, which has got 99% identity with *P.chlamydosoria* (AY593965.1) as indicated in Fig no.3 and 98% identity with *Verticillium chlamydosporium* (AY642328.1) as indicated in Fig no.4.

5.1.8 rDNA based strainal variation studies at ITS and IGS region of *Pochonia chlamydospora*.

Primers ITS-4 and ITS-5 amplified the ITS regions in all the isolates of *P.chlamydosoria*. The ITS primers generated a single band of 600bp. This size corresponded to the expected size of ITS region. Amplification of IGS region gave 500bp band size on 1% agarose gel as depicted in plate no.9.
Table No.5.3 List of Primers used in the study

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Primer name</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primers used for the amplification of β-tubulin gene in <em>P.chlamydosporia</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Tub 1F</td>
<td>5’ TTT GCT TCT CAG TGT TC3’</td>
<td>Hirch <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>2.</td>
<td>Tub 1R</td>
<td>5’ATG CAA GAA AGC CTT GCG AC3’</td>
<td></td>
</tr>
<tr>
<td>Primers used for the amplification of ITS region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>ITS4</td>
<td>5’ TCC TGCGCT TAT TGA TAT GC3’</td>
<td>Arora <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>6.</td>
<td>ITS5</td>
<td>5’ GGA AGT AAA AGT TCA AAG G3’</td>
<td></td>
</tr>
<tr>
<td>Primers used for the amplification of IGS region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>5SA</td>
<td>5’CAG AGT CCTATG GCC GTA AAT3’</td>
<td>Arora</td>
</tr>
<tr>
<td>8.</td>
<td>CNL12</td>
<td>5’CTGAAC GCT CCT GGG GAT TCAC3’</td>
<td><em>et al.</em> (1996)</td>
</tr>
</tbody>
</table>

Table No.5.4 Percent identity of sequenced β-tubulin gene with other existing sequences of Genbank.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Accession Number</th>
<th>Organism</th>
<th>% Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AY642328.1</td>
<td><em>Verticillium chlamydosporium</em></td>
<td>98%</td>
</tr>
<tr>
<td>2.</td>
<td>AY593965.1</td>
<td><em>Pochonia chlamydosporia</em></td>
<td>99%</td>
</tr>
<tr>
<td>3.</td>
<td>AY722412.1</td>
<td><em>Epichloe festucae</em></td>
<td>92%</td>
</tr>
<tr>
<td>4.</td>
<td>X52616.1</td>
<td><em>Epichloe typhina</em></td>
<td>92%</td>
</tr>
</tbody>
</table>

Thus the strains were identified as *P.chlamydosporia* based on the presence of β-tubulin gene.
5.2 To study the compatibility of *P. fluorescens* + *P. chlamydosporia* in *vitro* and in *vivo*.

5.2.1 Evaluating the Compatibility of *P. chlamydosporia* and *P. fluorescens* under *in vitro* conditions.

Compatibility of *P. chlamydosporia* (PC-3, PC-2) and *P. fluorescens* (PF-7) was evaluated under *in vitro* conditions in 3 sets of experimental designs (explained in experimental methods). Plate No. 10 indicates the compatibility of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) in the Set I of experimental design. Plate No. 11 indicates the compatibility of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) in the Set II of experimental design and Plate No. 12 indicates the compatibility of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) in the Set III of experimental design. Plate no. 13 indicates the compatibility of *P. chlamydosporia* (PC-2) and *P. fluorescens* (PF-7) in the Set III of experimental design. The results of each set of experiment were tabulated in Table No.5.5 in terms of % compatibility:

Over all % compatibility was calculated by the mean (average) of the pooled up average % compatibility found from each set of experiment. Over all % compatibility was found to be highest between PC-3 strain of *P. chlamydosporia* and PF-7 strain of *P. fluorescens*. Table No. 5.5
Table No. 5.5 Percent compatibility of *P. chlamydosporia* and *P. fluorescens* (PF-7) *in vitro* – Dual culture methods.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average % compatibility from set -I (Half plate method)</th>
<th>Average % compatibility from set -II (Cross streak method)</th>
<th>Average % compatibility from set -III (Streak plate method)</th>
<th>Overall % Compatibility from Set 1-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-3 + PF-7</td>
<td>80.87 (64.01)</td>
<td>85.67 (67.70)</td>
<td>87.45 (69.21)</td>
<td>84.66 (66.89)</td>
</tr>
<tr>
<td>PC-2 + PF-7</td>
<td>84.67 (66.89)</td>
<td>79.80 (63.29)</td>
<td>80.25 (63.58)</td>
<td>81.57 (64.52)</td>
</tr>
</tbody>
</table>

The overall average % compatibility between PC-3 + PF-7 was **84.66 %** followed by PC-2 + PF-7 which was **81.57 %**. The Fig no.5 indicates the percent compatibility *P. chlamydosporia* (PC-3 & PC-2) and *P. fluorescens* (PF-7) *in vitro*.

**Fig No: 5 Percent Compatibility of *P. chlamydosporia* (PC-3 & PC-2) and *P. Fluorescens* (PF-7) *in vitro***
Plate No.10 Compatibility of *P. fluorescens* (PF-7) + *P. chlamydosporia* (PC-3) under *in vitro* conditions by half plate

Control Plate of *P. chlamydosporia* (PC-3)  Control Plate of *P. fluorescens* (PF-7)

Control Plate of *P. chlamydosporia* (PC-3)  Plate indicating the compatibility between (PC-3) and (PF-7)
Plate No.11 Compatibility of *P. fluorescens* (PF-7) + *P. chlamydosporia* (PC-3) under *in vitro* conditions. II SET

Control Plate of (PC-3) Plate indicating the Compatibility between (PC-3 and PF-7) Control Plate of (PF-7)
Plate No.12 Compatibility of *P. fluorescens* (PF-7) & *P. chlamydosporia* (PC-3) under *in vitro* conditions III SET

Plate indicating the compatibility between *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7)

Control plate of *P. chlamydosporia* (PC-3)

Control plate of *P. fluorescens* (PF-7)
Plate No. 13 Compatibility of *P. fluorescens* (PF-7) + *P. chlamydosporia* (PC-2) under *in vitro* conditions

Plate indicating the compatibility between *P. chlamydosporia* (PC-2) and *P. fluorescens* (PF-7)

Control plate of *P. chlamydosporia* (PC-2)

Control plate of *P. fluorescens* (PF-7)
5.2.1.1 Evaluating the compatibility of *P. fluorescens* and *P. chlamydosporia* by mycelial dry weight method:

The compatibility of *P. chlamydosporia* (PC-3, PC-2) and *P. fluorescens* (PF-7) was evaluated under *in vitro* conditions by the mycelia dry weight method. The compatibility of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) can be seen in plate no.15 and plate no.16 indicates the compatibility between *P. chlamydosporia* (PC-2) and *P. fluorescens* (PF-7). In this experiment dry weight of fungal mat grown on the surface of PDB in the conical flask and Colony forming units of both the bio-agents at the time of completion of experiment was considered as parameters and tabulated in Tables no. 5.6.

**Plate No. 14  Compatibility of *P. chlamydosporia* and *P. fluorescens* in vitro by mycelia dry weight method**
Table No. 5.6  Percent compatibility of *P. chlamydosporia* (PC-3 – PC-2) and *P. fluorescens* (PF-7) *in vitro* – dry mycelial weight method

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry weight of mycelial mat (g)</th>
<th>% Increase in growth of <em>P. chlamydosporia</em></th>
<th>% Compatibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-3+PF</td>
<td>2.519667</td>
<td>64%</td>
<td>91.67%</td>
</tr>
<tr>
<td>PC-2+PF</td>
<td>1.233667</td>
<td>8.32%</td>
<td>86.93%</td>
</tr>
<tr>
<td>PC-3</td>
<td>2.898667</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>PC-2</td>
<td>1.345667</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>PF-7</td>
<td>1.119667</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

Fig No. 6 Percent Compatibility of *P. chlamydosporia* (PC-3 and PC-2) + *P. fluorescens* (PF-7) by mycelial dry weight method.
Table No. 5.7 CFU of *P. chlamydosporia* from the broth culture in the mycelia dry weight experiment:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>CFU (x10^8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC+PF</td>
<td>3.31</td>
<td>3.26</td>
<td>3.29</td>
<td>3.28</td>
</tr>
<tr>
<td>PC-2+PF</td>
<td>2.57</td>
<td>2.53</td>
<td>2.51</td>
<td>2.50</td>
</tr>
<tr>
<td>CONTROL</td>
<td>0.047</td>
<td>0.049</td>
<td>0.043</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Fig No. 7 Colony forming Units of *P.chlamydosporia* (PC-3 & PC-2) from the broth culture in the mycelial dry weight experiment.
Table No. 5.8 CFU of *P. fluorescens* in mycelia dry weight method

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>CFU (x10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC+PF</td>
<td>2.25</td>
<td>2.30</td>
<td>2.20</td>
<td>2.23</td>
</tr>
<tr>
<td>PC-2+PF</td>
<td>1.74</td>
<td>1.69</td>
<td>1.72</td>
<td>1.70</td>
</tr>
<tr>
<td>CONTROL</td>
<td>0.052</td>
<td>0.050</td>
<td>0.057</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Fig No. 8 Colony forming Units of *P. fluorescens* (PF-7) from the broth in the mycelial dry weight experiment.
Conical Flask containing the dual culture of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) indicates the compatibility as well as conical flasks alone with culture of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) serving as control.
Conical Flask containing the dual culture of *P. chlamydosporia* (PC-2) and *P. fluorescens* (PF-7) indicates the compatibility.
5.2.1.2 Evaluating the efficacy of *P. chlamydosporia* and *P. fluorescens* on the target fungal pathogen *F. oxysporum* f.sp. *vasinfectum*

Evaluated the bio-efficacy of *P. chlamydosporia* and *P. fluorescens* against *F. oxysporum* f.sp.*vasinfectum in vitro* by dual culture methods as explained in Table No.5.9. The % inhibition was calculated and the average values from each treatment were tabulated below. Final overall % inhibition was calculated by taking the means of (averages) all treatments. The inhibition in the radial growth of *F. oxysporum* f. sp. *vasinfectum* (FUS) by *P. chlamydosporia* (PC-3) can be clearly seen Plate no.17 and in Plate no.18 the inhibition in the radial growth of *F. oxysporum* f. sp. *vasinfectum* (FUS) by *P. chlamydosporia* (PC-2) can be seen. In Plate no.19 the inhibition in the radial growth of *F. oxysporum* f. sp. *vasinfectum* (FUS) by both *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) is clearly indicated and in Plate no.20 the inhibition in the radial growth of *F. oxysporum* f. sp. *vasinfectum* (FUS) by both *P. chlamydosporia* (PC-2) and *P. fluorescens* (PF-7) is clearly indicated.

**Table No. 5.9 Percent inhibition of *F. oxysporum* f. sp. *vasinfectum* (FUS) by *P. chlamydosporia* (PC-3 and PC-2) and *P. fluorescens* (PF-7) in vitro**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1.865</td>
<td>1.957</td>
<td>2.114</td>
<td>68.27 (55.67)</td>
</tr>
<tr>
<td>T2</td>
<td>2.512</td>
<td>1.738</td>
<td>1.923</td>
<td>47.08 (43.28)</td>
</tr>
<tr>
<td>T3</td>
<td>1.944</td>
<td>1.731</td>
<td>1.654</td>
<td>96.82 (79.69)</td>
</tr>
<tr>
<td>T4</td>
<td>2.142</td>
<td>2.821</td>
<td>3.252</td>
<td>94.47 (76.31)</td>
</tr>
</tbody>
</table>
Final overall % inhibition of *F. oxysporum* f.sp.*vasinfectum* by *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) *in vitro* was found to be 94.47%.

**Fig. No. 9 Percent inhibition of *F. oxysporum* f. sp.*vasinfectum* (FUS) by *P. chlamydosporia* (PC-3 and PC-2) and *P. fluorescens* (PF-7) *in vitro***

<table>
<thead>
<tr>
<th></th>
<th>PC-2+PF+FUS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>T5</td>
<td>1.99</td>
<td>1.78</td>
<td>1.783</td>
<td>62.37 (52.12)</td>
</tr>
<tr>
<td>T6</td>
<td>CONTROL</td>
<td>---</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

The Fig no.9 reveals the percentage inhibition in the growth of *F. oxysporum* f.sp.*vasinfectum* (FUS) by the bio-agent cultures *P. chlamydosporia* (PC-3) and *P. chlamydosporia* (PC-2) and *P. fluorescens* (PF-7) individually and also in combination *in vitro*. From the data is very evident that the combination of *P. chlamydosporia* (PC-3) and *P. fluorescens*
(PF-7) was very effective in inhibiting the growth of

*F. oxysporum f.sp. vasinfectum* (FUS).
Plate No.17 Inhibition of *F. oxysporum* f. sp. *vasinfectum* (FUS) by *P. chlamydospora* (PC-3) in vitro

Petri plate indicating the inhibition in the growth of *F. oxysporum* f. sp. *vasinfectum* (FUS) by *P. chlamydospora* (PC-3).

Petri plates inoculated with *P. chlamydospora* (PC-3) - CONTROL plates.

Petri plates inoculated with of *F. oxysporum* f. sp. *vasinfectum* (FUS) - CONTROL plates.
Plate No.18 Inhibition of *F. oxysporum* f. sp. *vasinfectum* by *P. chlamydosporia* (PC-2) *in vitro*

Petri plate indicating the inhibition in the growth of *F. oxysporum* f. sp. *vasinfectum* (FUS) by *P. chlamydosporia* (PC-2).

Petri plates innoculated with *P. chlamydosporia* (PC-2). CONTROL plates.

Petri plates innoculated with of *F. oxysporum* f. sp. *vasinfectum* (FUS) - CONTROL plates.
Plate No.19  Inhibition of \textit{F. oxysporum} f.sp.\textit{vasinfectum} by \textit{P. chlamydosporia} (PC-2) and \textit{P. fluorescens} (PF-7) in vitro.

Petri plate indicating the inhibition in the growth of \textit{F. oxysporum} f.sp.\textit{vasinfectum} (FUS) by \textit{P. chlamydosporia} (PC-2).

Petri plate indicating the inhibition in the growth of \textit{F. oxysporum} f.sp.\textit{vasinfectum} (FUS) by \textit{P. chlamydosporia} (PC-2) and \textit{P. fluorescens} (PF-7).
Plate No. 20 Inhibition of *F. oxysporum* f. sp.*vasinfectum* by *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) in vitro

Petri plate containing the dual culture of *F. oxysporum* f. sp.*vasinfectum* (FUS) and *P. fluorescens* (PF-7)

Petri plate indicating the inhibition in the growth of *F. oxysporum* f. sp.*vasinfectum* (FUS) by *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7)

Petri plate indicating the inhibition in the growth of *F. oxysporum* f. sp.*vasinfectum* (FUS) by *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7)
5.2.1.3 Evaluating the efficacy of *P. chlamydosporia* and *P. fluorescens* on the target fungal pathogen *F. oxysporum f.sp. vasinfectum* by dry mycelia method:

The efficacy of *P. chlamydosporia* and *P. fluorescens* in inhibiting the growth of the target fungal pathogen *F. oxysporum f.sp. vasinfectum* was evaluated by conducting mycelial dry weight experiment. This particular experiment was carried out in order to confirm the results of the above experiments. The experiment clearly indicates an inhibition in the growth of the pathogenic fungus *F. oxysporum f.sp. vasinfectum* by the bio-agents. Plate no. 21 indicates the inhibition in the growth of *F. oxysporum f.sp. vasinfectum* individually by *P. fluorescens* (PF-7). Plate no. 22 indicates the inhibition in the growth of *F. oxysporum f.sp. vasinfectum* (FUS) individually by *P. chlamydosporia* (PC-3) and Plate no. 23 indicates the inhibition in the growth of *F. oxysporum f.sp. vasinfectum* (FUS) in combination by *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7). Plate no. 24 indicates the inhibition in the growth of *F. oxysporum f.sp. vasinfectum* (FUS) individually by *P. chlamydosporia* (PC-2) and Plate no. 26 indicates the inhibition in the growth of *F. oxysporum f.sp. vasinfectum* (FUS) in combination by *P. chlamydosporia* (PC-2) and *P. fluorescens* (PF-7). The dried fungal mats of *P. chlamydosporia* (PC-3), *P. chlamydosporia* (PC-2) and *F. oxysporum f.sp. vasinfectum* (FUS) in the filter paper are seen in Plate no. 27.
Plate No. 21 Efficacy of *P. fluorescens* (PF-7) against the target pathogen i.e *F. oxysporum* f.sp. *vasinfectum*

Conical Flask containing culture of *P. fluorescens* (PF-7) and *F. oxysporum* f. sp. *vasinfectum* (FUS) as well as dual cultures of *P. fluorescens* (PF-7) *F. oxysporum* f. sp. *vasinfectum* (FUS)

Plate No. 22 Efficacy of *P. chlamydosporia* (PC-3) against the target pathogen i.e *F. oxysporum* f.sp. *vasinfectum*
Conical Flasks containing cultures of *P. chlamydosporia* (PC-3), *F. oxysporum* f. sp. *vasinfectum* (FUS) and *P. fluorescens* (PF-7).
Conical Flask containing culture of *P. chlamydospora* (PC-2) and *F. oxysporum* f. sp. *vasinfectum* (FUS) as well as dual cultures of *P. chlamydospora* (PC-2) and *F. oxysporum* f. sp. *vasinfectum* (FUS).

Plate No. 24 Efficacy of *P.chlamydospora* (PC-2) against the target pathogen i.e *F. oxysporum* f.sp.*vasinfectum*

Conical Flask containing culture of *P. fluorescens* (PF-7) and *F. oxysporum* f. sp. *vasinfectum* (FUS) as well as dual cultures of *P. fluorescens* (PF-7) *F. oxysporum* f. sp. *vasinfectum* (FUS).

Plate No. 25 Efficacy of *P. fluorescens* against the target pathogen i.e *F. oxysporum* f.sp. *vasinfectum*
Conical Flasks containing cultures of *P. chlamydosporia* (PC-2), *F. oxysporum* f. sp. *vasinfectum* (FUS) and *P. fluorescens* (PF-7).

Plate No. 26 Efficacy of *P. fluorescens* (PF-7) + *P. chlamydosporia* (PC-2) against the target pathogen i.e *Fusarium oxysporum*

Filter paper containing dried fungal mats of *P. chlamydosporia* (PC-3), (PC-2) and *F. oxysporum* f. sp. *vasinfectum* (FUS)

Plate No.27 Dried Fungal Mats
5.2.1.4 Evaluating the compatibility of *P. chlamydosporia* and *P. fluorescens* under *in vivo* conditions.

The results of evaluation of compatibility of two strains of *P. chlamydosporia* (PC-3 and PC-2) and *P. fluorescens* *in vitro* has shown PC-3 + PF-7 as the best compatible treatment of combination of these bio-agents *in vivo* also, from the plant growth parameters explained in (Table No. 5.10) and extent of root colonization presented in (Table No. 5.11). The growth differences in the tomato plants due the various treatments are clearly shown, it also indicates that the treatment PC-3+PF-7 is the best among all in Plate no.28. The difference in the root mass due the various treatments is clearly shown in Plate no.29.
Plate No. 28 Evaluation of compatibility of *P. chlamydosporia* (PC-3 and PC-2) with *P. fluorescens* (PF-7) *in vivo*

**A**: PC-3 (SD+SB); **B**: PC-2 (SD+SB); **C**: PF-7 (SD+SB); **D**: PC-3+PF-7 (SD+SB); **E**: PC-2+PF-7 (SD+SB); **F**: CONTROL
Plate No.29  Roots of tomato indicating the compatibility between *P. chlamydosporia* (PC-3 and PC-2) and *P. fluorescens* (PF-7).

Roots indicating the treatmental differences.
Table No.5.10 Plant Growth Parameter of the compatibility experiment of *P. chlamydospora* (PC-3 and PC-2) and *P. fluorescens* (PF-7)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root Length (cm)</th>
<th>Shoot Length (cm)</th>
<th>Root Weight (g)</th>
<th>Shoot Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 PC-3(SD+SB)</td>
<td>10.65</td>
<td>25.18</td>
<td>2.68</td>
<td>7.84</td>
</tr>
<tr>
<td>T2 PC-2(SD+SB)</td>
<td>10.55</td>
<td>25.10</td>
<td>2.22</td>
<td>9.2</td>
</tr>
<tr>
<td>T3 PF-7(SD+SB)</td>
<td>10.65</td>
<td>24.76</td>
<td>2.68</td>
<td>9.94</td>
</tr>
<tr>
<td>T4 PC-3+PF-7(SD+SB)</td>
<td>13.98</td>
<td>28.06</td>
<td>4.86</td>
<td>10.72</td>
</tr>
<tr>
<td>T5 PC-2+PF-7 (SD+SB)</td>
<td>13.11</td>
<td>27.03</td>
<td>3.76</td>
<td>9.14</td>
</tr>
<tr>
<td>T6 CONTROL</td>
<td>8.36</td>
<td>23.09</td>
<td>2.13</td>
<td>8.34</td>
</tr>
<tr>
<td>CD -5%</td>
<td>1.46</td>
<td>2.78</td>
<td>0.72</td>
<td>1.25</td>
</tr>
</tbody>
</table>

**Fig No. 10 Plant Growth parameter indicating the compatibility between *P. chlamydospora* (PC-3) and *P. fluorescens* (PF-7).**

From the Fig No.10 it is very evident that the combination of *P. chlamydospora* (PC-3) and *P. fluorescens* (PF-7) in compatibility experiment *in vivo* in tomato was the best and also very effective in increasing the plant growth parameters.
Table No. 5.11 Extent of root colonization (CFU) of *P. chlamydosporia* (PC-3 and PC-2) and *P. fluorescens* (PF-7) in compatibility experiment *in vivo* in tomato.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CFU of <em>P. chlamydosporia</em> (× 10⁶) / 1g root</th>
<th>CFU of <em>P. fluorescens</em> (× 10⁷) / 1g root</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 PC-3(SD+SB)</td>
<td>2.316</td>
<td>----</td>
</tr>
<tr>
<td>T2 PC-2(SD+SB)</td>
<td>1.390</td>
<td>----</td>
</tr>
<tr>
<td>T3 PF-7(SD+SB)</td>
<td>----</td>
<td>1.7414</td>
</tr>
<tr>
<td>T4 PC-3+PF-7</td>
<td>2.990</td>
<td>3.690</td>
</tr>
<tr>
<td>T5 PC-2+PF-7</td>
<td>2.215</td>
<td>2.701</td>
</tr>
<tr>
<td>T6 CONTROL</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD -5%</td>
<td>0.78</td>
<td>0.84</td>
</tr>
</tbody>
</table>

All the figures are the mean (average) values of 5 replicates.
5.3 Development of the combination formulation of *P. fluorescens* + *P. chlamydosporia* and evaluate the shelf life.

The strain of both *P. fluorescens* and *P. chlamydosporia* which proved effective in controlling *F. oxysporum* f.sp.*vasinfectum* and *M. incognita* respectively were used for developing combination formulation.

Protocol was standardized for the development of solid and liquid formulation. Solid formulation was standardized by mixing the bio-agents in talc powder and CMC (carboxy methyl cellulose).

The shelf life of combination formulation of *P. fluorescens* + *P. chlamydosporia* was evaluated by taking CFU (colony forming units) over a period of 15 months. The results indicated maximum CFU during the first three months of development of formulation. The CFU gradually reduced after 8 to 9 months, but still the CFU indicated the presences of few good no. of colonies of both the organisms even after 15 months.

The CFU taken over a period of 15 months are tabulated in the Table No.5.12. Plate no.30 depicts the evaluation of shelf life after six months of packaging. In this plate good no. of colonies of *P. fluorescens* *P. chlamydosporia* were seen with the CFU of *P. chlamydosporia* being 1.8 x $10^8$/g and CFU of *P. fluorescens* 3.4 x $10^9$/g. Plate no.31 depicts the evaluation of shelf life after nine months of packaging. After nine months of packing also colonies of *P. fluorescens* and *P. chlamydosporia* can be clearly seen with the CFU of *P. chlamydosporia* being 5.2 x $10^7$/g and CFU of *P. fluorescens* 1.2 x $10^9$/g. Whereas Plate no.32 indicates the evaluation of
shelf life after twelve months of packaging with the CFU of
*P. chlamydosporia* being $1.81 \times 10^7$/g and CFU of *P. fluorescens* $6.3 \times 10^8$/g

**Table No. 5.12 CFU data on shelf life of *P. chlamydosporia* + *P. fluorescens* for 15 Months**

<table>
<thead>
<tr>
<th>Duration in months</th>
<th>CFU of <em>P. chlamydosporia</em>/g</th>
<th>CFU of <em>P. fluorescens</em>/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>I month</td>
<td>$8.4 \times 10^8$</td>
<td>$9.3 \times 10^9$</td>
</tr>
<tr>
<td>II month</td>
<td>$7.2 \times 10^8$</td>
<td>$7.7 \times 10^9$</td>
</tr>
<tr>
<td>III month</td>
<td>$6.0 \times 10^8$</td>
<td>$5.6 \times 10^9$</td>
</tr>
<tr>
<td>IV month</td>
<td>$5.2 \times 10^8$</td>
<td>$4.3 \times 10^9$</td>
</tr>
<tr>
<td>V month</td>
<td>$3.0 \times 10^8$</td>
<td>$3.7 \times 10^9$</td>
</tr>
<tr>
<td>VI month</td>
<td>$1.8 \times 10^8$</td>
<td>$3.4 \times 10^9$</td>
</tr>
<tr>
<td>VII month</td>
<td>$8.4 \times 10^7$</td>
<td>$2.6 \times 10^9$</td>
</tr>
<tr>
<td>VIII month</td>
<td>$6.8 \times 10^7$</td>
<td>$2.0 \times 10^9$</td>
</tr>
<tr>
<td>IX month</td>
<td>$5.2 \times 10^7$</td>
<td>$1.2 \times 10^9$</td>
</tr>
<tr>
<td>X month</td>
<td>$4.0 \times 10^7$</td>
<td>$9.4 \times 10^8$</td>
</tr>
<tr>
<td>XI month</td>
<td>$3.1 \times 10^7$</td>
<td>$8.2 \times 10^8$</td>
</tr>
<tr>
<td>XII month</td>
<td>$1.81 \times 10^7$</td>
<td>$6.3 \times 10^8$</td>
</tr>
<tr>
<td>XIII month</td>
<td>$9.7 \times 10^6$</td>
<td>$5.6 \times 10^8$</td>
</tr>
<tr>
<td>XIV month</td>
<td>$7.5 \times 10^6$</td>
<td>$5.0 \times 10^8$</td>
</tr>
<tr>
<td>XV month</td>
<td>$5.6 \times 10^6$</td>
<td>$3.6 \times 10^8$</td>
</tr>
</tbody>
</table>
Plate No.30 Shelf Life evaluation of the combination formulation of *P. fluorescens* (PF-7) + *P. chlamydosporia* (PC-3) - I

Evaluation of shelf life after Six Months of Packaging
Plate No.31 Shelf Life Evaluation of the combination formulation *P. fluorescens* (PF-7) + *P. chlamydospora* (PC-3) - II

Evaluation of shelf life after Nine Months of Packaging
Plate No.32 Shelf Life Evaluation of the combination formulation *P. fluorescens* (PF-7) + *P. chlamydosporia* (PC-3) - III

Evaluation of shelf life after Twelve Months of Packaging
5.4 Evaluation of the bio-efficacy of *P. fluorescens* and *P. chlamydosporia* against *M. incognita* and *F. oxysporum* f.sp.*vasinfectum* under screen house conditions.

Plant growth parameters and % disease incidence were observed at the end of the experiment and observations were recorded. The results were tabulated for the bio-agent treated plants inoculated with *M. incognita*, *F. oxysporum* f.sp.*vasinfectum* and with both pathogens separately in the table respectively, showing the mean averages of 5 replicates.

The observations on plant growth parameters, extent of root colonization by means of CFU from 1g of root, root galling index based on 0.1-10 scale, no. of J2/10g of root, no. of J2/100 cc of soil, were presented.

From the observations given in the Table 5.15 it is evident that the combination formulations of *P. chlamydosporia* and *P. fluorescens* enriched with neem cake T9-[PF+PC+NC (SD+SB)] has shown better results in terms of all the parameters observed in case of infestation with single pathogen alone and also in case of infestation with both the pathogens. The treatment has not only boosted the plant growth but also brought down the nematode population. The shoot weight was recorded the heighest in this treatment being 61.1 cm, shoot weight being 26.7g, followed by root weight of 9.3g and root length of 24.1cm. The no. of J2/g of root being 23 and no. of J2/100 cc of soil being 36. Disease incidence to the tune 15% was observed. Fig No. 15 indicates the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched neem cake on crop growth of okra. Fig No.16 indicates the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched neem cake on reduction of *M. incognita*
population and reduction of disease incidence under screen house conditions.

The Table No.5.13 indicates the **effect of combination of** *P. chlamydosporia* and *P. fluorescens* **enriched Vermicompost** on crop growth of Okra under screen house conditions. **Table No.5.14** indicates the **effect of combination of** *P. chlamydosporia* and *P. fluorescens* **enriched FYM** on crop growth of Okra under screen house conditions.

In Plate no.33 one can clearly see the growth differences in the okra plant brought about by the combination of *P. chlamydosporia* and *P. fluorescens* being enriched in three different organic substrates viz Neem cake, FYM and vermicompost under screen house conditions. The growth of okra plants was more vigorous and healthy in the treatment where the combination of *P. chlamydosporia* and *P. fluorescens* were enriched in neem cake.

In Plate No.35 one can see the effect of *P. fluorescens* (PF-7) on nematode eggs. In the treatment control the nematodes were well developed in there respective egg shell when compared to the eggs in the treatment with *P. fluorescens* (PF-7). The nematodes in this treatment were seen distorted with formation of granular structures inside them.

In Plate No.36 one can see the effect of *P. chlamydosporia* (PC-3) on nematode eggs. This treatment clearly indicates the nematophagous property of *P.chlamydosporia*. In this treatment we were able to observe that the
development of nematode in the eggshell was distorted due to the chlamydospores penetration into the eggshell.

Plate No.37 indicates the effect of combination of *P.chlamydosporia* (PC-3) and *P.fluorescens* (PF-7) on nematode egg-masses. Since this treatment involved combination of *P.chlamydosporia* and *P. fluorescens* (PF-7) we were able to clearly see the nematophaous property of *P.chlamydosporia*, we were also able to see the effect of *P. fluorescens* (PF-7) in the formation of granular structure inside the nematodes.

Plate No: 38 indicate the effect of combination of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Neem Cake (NC), under Screen house conditions. This plate indicates the the roots of okra uprooted during the termination of the experiment. The experiment was conducted under screen house condition to see the effect of combination of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched with Neem Cake (NC).

In the plate Photograph A reveals the Control roots of okra v/s root of okra from the treatment where both the seed and substrate have been treated with combination formulation of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) with enriched Neem Cake.

In the plate Photograph B depicts the Control roots of okra v/s root of okra from the treatment where both the seed and substrate have been treated with only *P. chlamydosporia* (PC-3) formulation with enriched Neem Cake.

In the plate Photograph C depicts the Control roots of okra v/s root of okra from the treatment where both the seed and substrate have been
treated with only *P. fluorescens* (PF-7) formulation with enriched Neem Cake.
Table No. 5.13 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Vermicompost on crop growth of Okra under screen house conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot Length (cm)</th>
<th>Shoot Wt. (g)</th>
<th>Root Length (cm)</th>
<th>Root Wt. (g)</th>
<th>Gall index (1-10)</th>
<th>No. of J2 in 10g root</th>
<th>No. of J2 in 100cc soil</th>
<th>CFU of <em>P. chlamydosporia</em> (x 10^6) / 1g root</th>
<th>CFU of <em>P. fluorescens</em> (x 10^7) / 1g root</th>
<th><em>% disease incidence</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>51</td>
<td>21.5</td>
<td>20.52</td>
<td>5.24</td>
<td>6.6</td>
<td>34</td>
<td>75</td>
<td>0</td>
<td>3.5</td>
<td>37 (37.47)</td>
</tr>
<tr>
<td>T2</td>
<td>50.52</td>
<td>20.44</td>
<td>16.38</td>
<td>5.92</td>
<td>5.6</td>
<td>37</td>
<td>59</td>
<td>0</td>
<td>4.0</td>
<td>30 (33.21)</td>
</tr>
<tr>
<td>T3</td>
<td>52.66</td>
<td>21.68</td>
<td>17.08</td>
<td>6.02</td>
<td>5.3</td>
<td>39</td>
<td>46</td>
<td>0</td>
<td>4.8</td>
<td>28 (31.95)</td>
</tr>
<tr>
<td>T4</td>
<td>49.18</td>
<td>19.02</td>
<td>15.66</td>
<td>5.22</td>
<td>7.4</td>
<td>35</td>
<td>68</td>
<td>2.6</td>
<td>0</td>
<td>44 (41.55)</td>
</tr>
<tr>
<td>T5</td>
<td>50.54</td>
<td>19.06</td>
<td>16.7</td>
<td>5.02</td>
<td>5.9</td>
<td>32</td>
<td>58</td>
<td>3.7</td>
<td>0</td>
<td>41 (39.82)</td>
</tr>
<tr>
<td>T6</td>
<td>51.42</td>
<td>21.4</td>
<td>18.04</td>
<td>5.23</td>
<td>5.5</td>
<td>46</td>
<td>66</td>
<td>4.2</td>
<td>0</td>
<td>35 (36.27)</td>
</tr>
<tr>
<td>T7</td>
<td>53.8</td>
<td>22.58</td>
<td>18.98</td>
<td>6.56</td>
<td>5.3</td>
<td>54</td>
<td>61</td>
<td>2.9</td>
<td>2.7</td>
<td>31 (33.83)</td>
</tr>
<tr>
<td>T8</td>
<td>58.02</td>
<td>22.98</td>
<td>19.76</td>
<td>6.07</td>
<td>5.0</td>
<td>41</td>
<td>51</td>
<td>3.8</td>
<td>2.9</td>
<td>26 (30.66)</td>
</tr>
<tr>
<td>T9</td>
<td>60.07</td>
<td>23.3</td>
<td>21.28</td>
<td>7.4</td>
<td>4.5</td>
<td>30</td>
<td>40</td>
<td>4.5</td>
<td>3.4</td>
<td>17 (24.35)</td>
</tr>
<tr>
<td>T10</td>
<td>40.3</td>
<td>16.04</td>
<td>12.62</td>
<td>4.94</td>
<td>8.9</td>
<td>72</td>
<td>143</td>
<td>0</td>
<td>0</td>
<td>46 (42.71)</td>
</tr>
<tr>
<td>CD-5%</td>
<td>3.64</td>
<td>1.05</td>
<td>1.14</td>
<td>0.55</td>
<td>0.76</td>
<td>1.46</td>
<td>7.24</td>
<td>0.49</td>
<td>0.34</td>
<td>---</td>
</tr>
</tbody>
</table>

*T1: PF-SD; T2: PF+VER-SB; T3: PF+VER (SD+SB); T4: PC-SD; T5: PC+VER-SB; T6: PC+VER (SD+SB) T7: PC+PF-SD; T8: PC+PF+VER-SB T9: PF+PC+VER (SD+SB) T10: CONTROL [SD: seed treatment; SB: substrate treatment; VER: vermicompost]*
Fig no.11 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with vermicompost on crop growth of Okra.

![Graph showing effect of combination of P. chlamydosporia and P. fluorescens enriched with vermicompost on crop growth of Okra.](image)

Fig No.12 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with vermicompost on reduction of *M. incognita* and reduction of disease incidence.

![Graph showing effect of combination of P. chlamydosporia and P. fluorescens enriched with vermicompost on reduction of M. incognita and reduction of disease incidence.](image)
Table No.5.14 Effect of combination of *P. chlamydosporia* and *P. fluorescens* with enriched FYM on crop growth of Okra under Screen house conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot length (cm)</th>
<th>Shoot wt.(g)</th>
<th>Root length (cm)</th>
<th>Root wt.(g)</th>
<th>Gall index (1-10)</th>
<th>No. of J2 in 10g root</th>
<th>No. of J2 in 100cc soil</th>
<th>CFU of <em>P. chlamydosporia</em> (x 10^6) / 1g root</th>
<th>CFU of <em>P. fluorescens</em> (x 10^7) / 1g root</th>
<th>% disease incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>50.5</td>
<td>21.46</td>
<td>19.72</td>
<td>6.12</td>
<td>6.8</td>
<td>36</td>
<td>79</td>
<td>0</td>
<td>2.7</td>
<td>34 (35.67)</td>
</tr>
<tr>
<td>T2</td>
<td>51.64</td>
<td>23.77</td>
<td>20.38</td>
<td>7.88</td>
<td>5.7</td>
<td>40</td>
<td>65</td>
<td>0</td>
<td>3.3</td>
<td>25 (30.00)</td>
</tr>
<tr>
<td>T3</td>
<td>53.06</td>
<td>24.02</td>
<td>22.04</td>
<td>8.2</td>
<td>5.2</td>
<td>55</td>
<td>64</td>
<td>0</td>
<td>3.0</td>
<td>27 (31.31)</td>
</tr>
<tr>
<td>T4</td>
<td>52.12</td>
<td>20.14</td>
<td>17.5</td>
<td>5.4</td>
<td>7.7</td>
<td>34</td>
<td>43</td>
<td>2.3</td>
<td>0</td>
<td>40 (39.23)</td>
</tr>
<tr>
<td>T5</td>
<td>53.62</td>
<td>21.0</td>
<td>17.78</td>
<td>6.06</td>
<td>6.1</td>
<td>43</td>
<td>55</td>
<td>3.4</td>
<td>0</td>
<td>35 (36.27)</td>
</tr>
<tr>
<td>T6</td>
<td>54.16</td>
<td>22.08</td>
<td>18.52</td>
<td>6.8</td>
<td>5.8</td>
<td>49</td>
<td>61</td>
<td>4.5</td>
<td>0</td>
<td>32 (34.45)</td>
</tr>
<tr>
<td>T7</td>
<td>52.22</td>
<td>23.06</td>
<td>17.8</td>
<td>7.14</td>
<td>5.5</td>
<td>42</td>
<td>69</td>
<td>2.8</td>
<td>3.8</td>
<td>28 (31.95)</td>
</tr>
<tr>
<td>T8</td>
<td>58.02</td>
<td>22.98</td>
<td>19.76</td>
<td>6.07</td>
<td>5.2</td>
<td>32</td>
<td>41</td>
<td>2.7</td>
<td>1.8</td>
<td>23 (28.66)</td>
</tr>
<tr>
<td>T9</td>
<td>58.68</td>
<td>21.74</td>
<td>20.96</td>
<td>6.33</td>
<td>4.6</td>
<td>34</td>
<td>42</td>
<td>3.8</td>
<td>2.6</td>
<td>16 (23.58)</td>
</tr>
<tr>
<td>T10</td>
<td>40.3</td>
<td>16.04</td>
<td>12.62</td>
<td>4.94</td>
<td>8.6</td>
<td>86</td>
<td>159</td>
<td>0</td>
<td>0</td>
<td>43 (40.98)</td>
</tr>
<tr>
<td>CD-5%</td>
<td>3.35</td>
<td>1.69</td>
<td>1.06</td>
<td>1.02</td>
<td>1.32</td>
<td>4.65</td>
<td>7.53</td>
<td>0.54</td>
<td>0.35</td>
<td>----</td>
</tr>
</tbody>
</table>

*T1*: PF-SD; *T2*: PF+FYM-SB; *T3*: PF+FYM(SD+SB); *T4*: PC-SD; *T5*: PC+FYM-SB; *T6*: PC+FYM(SD+SB) *T7*: PC+PF-SD; *T8*: PC+PF+FYM-SB

*T9*: PF+PC+FYM (SD+SB) *T10*: CONTROL  
*SD*: seed treatment; *SB*: substrate treatment; *FYM*: Farm Yard Manure

*log transformed values are not given in the table*
Fig No. 13 Effect of combination of *P. chlamydosporia* and *P. fluorescens* with enriched FYM on crop growth of Okra under screen house conditions.

![Bar chart showing the effect of combination of *P. chlamydosporia* and *P. fluorescens* with enriched FYM on crop growth of Okra under screen house conditions.](image)

Fig No. 14 Effect of combination of *P. chlamydosporia* and *P. fluorescens* with enriched FYM on reduction of *M. incognita* population and reduction of disease incidence

![Bar chart showing the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with FYM on reduction of *M. incognita* population and reduction of disease incidence under screen house conditions.](image)
Table No.5.15 Effect of combination of *P. chlamydosporia* and *P. fluorescens* with enriched Neem cake on crop growth of Okra under screenhouse conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot Length (cm)</th>
<th>Shoot Wt.(g)</th>
<th>Root Length (cm)</th>
<th>Root Wt.(g)</th>
<th>Gall index (1-10)</th>
<th>No. of J2 in 10g root</th>
<th>No. of J2 in 100cc soil</th>
<th>CFU of <em>P. chlamydosporia</em> ((x 10^6) / 1g) root</th>
<th>CFU of <em>P. fluorescens</em> ((x 10^7) / 1g) root</th>
<th>% disease incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>51.7</td>
<td>23.04</td>
<td>19.04</td>
<td>7.4</td>
<td>6.6</td>
<td>48</td>
<td>66</td>
<td>0</td>
<td>3.6</td>
<td>34 (35.67)</td>
</tr>
<tr>
<td>T2</td>
<td>52.3</td>
<td>24.8</td>
<td>23.6</td>
<td>8.1</td>
<td>5.5</td>
<td>46</td>
<td>68</td>
<td>0</td>
<td>4.8</td>
<td>22 (27.97)</td>
</tr>
<tr>
<td>T3</td>
<td>53.9</td>
<td>24.7</td>
<td>21.7</td>
<td>8.9</td>
<td>5.0</td>
<td>42</td>
<td>59</td>
<td>0</td>
<td>4.2</td>
<td>26 (30.66)</td>
</tr>
<tr>
<td>T4</td>
<td>50.0</td>
<td>22.3</td>
<td>15.2</td>
<td>7.6</td>
<td>7.6</td>
<td>37</td>
<td>70</td>
<td>3.1</td>
<td>0</td>
<td>38 (38.06)</td>
</tr>
<tr>
<td>T5</td>
<td>51.2</td>
<td>21.8</td>
<td>16.1</td>
<td>6.8</td>
<td>6.0</td>
<td>36</td>
<td>67</td>
<td>4.7</td>
<td>0</td>
<td>34 (35.67)</td>
</tr>
<tr>
<td>T6</td>
<td>52.3</td>
<td>24.3</td>
<td>16.3</td>
<td>7.5</td>
<td>5.5</td>
<td>39</td>
<td>58</td>
<td>3.9</td>
<td>0</td>
<td>30 (33.21)</td>
</tr>
<tr>
<td>T7</td>
<td>53.8</td>
<td>24.8</td>
<td>21.4</td>
<td>7.0</td>
<td>5.3</td>
<td>32</td>
<td>68</td>
<td>3.5</td>
<td>2.8</td>
<td>27 (31.31)</td>
</tr>
<tr>
<td>T8</td>
<td>59.3</td>
<td>25.3</td>
<td>20.5</td>
<td>8.4</td>
<td>5.1</td>
<td>33</td>
<td>53</td>
<td>4.6</td>
<td>3.0</td>
<td>20 (26.56)</td>
</tr>
<tr>
<td>T9</td>
<td>61.1</td>
<td>26.7</td>
<td>24.1</td>
<td>9.3</td>
<td>4.3</td>
<td>23</td>
<td>36</td>
<td>4.5</td>
<td>3.4</td>
<td>15 (22.79)</td>
</tr>
<tr>
<td>T10</td>
<td>41.9</td>
<td>19.2</td>
<td>14.9</td>
<td>8.5</td>
<td>8.6</td>
<td>67</td>
<td>135</td>
<td>0</td>
<td>0</td>
<td>45 (42.13)</td>
</tr>
<tr>
<td>CD-5%</td>
<td>4.82</td>
<td>2.44</td>
<td>1.25</td>
<td>0.61</td>
<td>0.56</td>
<td>1.58</td>
<td>3.26</td>
<td>0.26</td>
<td>0.32</td>
<td>----</td>
</tr>
</tbody>
</table>

*T1*: PF-SD; *T2*: PF+NC-SB; *T3*: PF+NC (SD+SB); *T4*: PC-SD; *T5*: PC+NC-SB; *T6*: PC+NC (SD+SB) *T7*: PC+PF-SD; *T8*: PC+PF+NC-SB
*T9*:PF+PC+NC (SD+SB) *T10*: CONTROL [SD: seed treatment; SB: substrate treatment; NC: Neem cake]
Fig No.15 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched neem cake on crop growth of okra

**Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with neem cake on crop growth of okra under Screen House Conditions**

- T1: PF-SD
- T2: PF+NC-SE
- T3: PF+NC (RT+RP)
- T4: PC-SD
- T5: PC+NC-SE
- T6: PC+NC (RT+RP)
- T7: PC+PF (H1)
- T8: PC+PF+NC-SE
- T9: PC+PF+NC (H1+UL)
- T10: CONTROL

Fig No.16 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched neem cake on reduction of *M. incognita* population and reduction of disease incidence under screen house conditions

**Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with Neem Cake on reduction of *M. incognita* population and reduction of disease incidence under screen house conditions.**

- T1: PF-SD
- T2: PF+NC-SE
- T3: PF+NC (SD+SB)
- T4: PC-SD
- T5: PC+NC-SE
- T6: PC+NC (SD+SB)
- T7: PC+PF-SD
- T8: PC+PF+NC-SE
- T9: PC+PF+NC (SD+SB)
- T10: CONTROL
Plate No. 33 Effect of combination of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Neem Cake, FYM and Vermicompost under screen house conditions.

Best Treatment: PF+PF+NC (SD+SB) Treatment: PF+PF+FYM (SD+SB)

Treatment: PF+PF+VER (SD+SB) Treatment: CONTROL
Plate No. 34 Effect of combination of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Neem Cake, FYM and Vermicompost under screen house conditions. - II
Plate No. 34 (a) Effect of combination of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Neem Cake, FYM and Vermicompost under screen house conditions. - II

Treatment: PF+PF+VER (SD+SB)

Treatment: CONTROL
Plate No.35 Effect of *P. fluorescens* (PF-7) on nematode eggs.
Plate No.36 Effect of *P. chlamydosporia* (PC-3) on nematode eggs

CONTROL

PC INFECTED NEMATODE EGGS
Plate No.37 Effect of combination of *P.chlamydosporia* (PC-3) and *P.fluorescens* (PF-7) on nematode egg-masses.
Plate No: 38 Effect of combination of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Neem Cake (NC), under Screen house conditions.

A: Control roots of okra v/s root of okra from the treatment where both the seed and substrate have been treated with combination formulation of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) with enriched Neem Cake.

B: Control roots of okra v/s root of okra from the treatment where both the seed and substrate have been treated with only *P. chlamydosporia* (PC-3) formulation with enriched Neem Cake.

C: Control roots of okra v/s root of okra from the treatment where both the seed and substrate have been treated with only *P. fluorescens* (PF-7) formulation with enriched Neem Cake.
5.5 Standardization of methods for the management of disease complex caused by *F. oxysporum* f.sp. *vasinfectum* + *M. incognita* on okra under field conditions.

The results for each set of experiments 4.4.1 A, B, C and D are tabulated as follows in two different tables separately for each set. The tables indicate the yield and % increase in yield and% reduction of disease incidence. Observations on plant growth parameters, extent of root colonization by means of CFU from 1g of root, root galling index based on 0.1-10 scale, no. of J2/10g of root, no. of J2/100 cc of soil, for each treatment recorded are indicated in the following tables.

The results indicate that treatment of seeds with both the bio-agents as well as application of neem enriched with formulation of *P. chlamydosporia* and *P. fluorescens* (T9-PF+PC-SD+SB) **proved significantly effective in the management of disease complex caused by *F. oxysporum* f.sp.*vasinfectum* and *M. incognita* in okra under field condition** (Table-5.16 and 5.17). This treatment reduced *M. incognita* infestation by 68% and disease incidence caused by *F. oxysporum* by 57%. Root colonization of *P. chlamydosporia* was found to be $4.9 \times 10^{-5}$ and *P. fluorescens* to be $6.7 \times 10^{-6}$ (Table No. 5.16). Table no. 5.18 indicates the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with FYM on crop growth of Okra under field conditions and Table No.5.19 indicates the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with FYM on root colonization and yield of okra under open field conditions. There was an increase in yield by 19.35%. Table No.5.20 indicates the effect of combination of *P. chlamydosporia* and
*P. fluorescens* enriched with vermicompost on crop growth of Okra under field conditions and Table No.5.19 indicates the effect of combination of *P. chlamydospora* and *P. fluorescens* enriched with vermicompost on root colonization and yield of okra under open field. There was an increase in yield by 19.35%.

The fungus is not an aggressive colonizer; it survives in soil for several months after a single application and may need repeated applications to enhance its multiplication in soil and is potential to reduce nematode density under field conditions (Jatala, 1986; Rao and Reddy, 1997c). Hence we enriched neem cake with this bio-agent and applied to soil. *P. chlamydospora* and *P. fluorescens* could be multiplied in neem cake enrichment process. Rao *et al.* (2003) also observed this phenomenon in their experiments. Further application of bio-agent through neem cake/vermicompost / FYM makes it easy to apply in the main field. The efficacy of the bio-agent under field conditions largely depends on delivery system and application methodology. Whatever the delivery system is adopted it should deliver the bio-agent to the rhizosphere of the crop. As soil is highly dynamic with various interactions among micro-organisms around the rhizosphere predominately to survive and establish in the root rhizosphere in the midst of interactions from pathogenic fungi / nematode or bacteria. **Integration of both the bio-agents in combination has proved to be significantly effective in increasing the yield of the crop by 28% (Table–5.17) conditions.**
Application of *P. fluorescens* was found to be effective against *M. incognita* (Santhi and Siva kumar, 1995) in tomato.

*P. fluorescens* is known to control the pathogen by the mechanism called siderophoric effect. It was also found very effective in the control of various soil borne fungi causing root rot in certain vegetables crops (Mukhopadhaya, 1987). Induction of growth promoting substances from the PGPR (Plant Growth Promoting rhizobacteria) and induction of systemic resistance against pathogenic fungi was reported by Ramamoorthy *et al.*, 2001 and in nematodes by Rao and Shylaja, 2004.

In general the growth of okra plants treated with *P. fluorescens* was better. This could be due to the plant growth promoting activity of *P. fluorescens* which is very well documented (Bloemberg and Lugtenberg, 2001; Kishore *et al.*, 2005).
Plate No.39 Standardization of methods for the management of disease complex caused by *F. oxysporum* f.sp.*vasinfectum* + *M. incognita* on okra under field conditions with bio-agent enriched Neem cake.

Overview of okra experimental plots in IIHR
Table No.5.16 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Neem cake on crop growth and nematode induced disease complex in okra under field conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot Length</th>
<th>Shoot Wt.(g)</th>
<th>Root Length</th>
<th>Root Wt.(g)</th>
<th>Root galling index on 1-10 Scale</th>
<th>Reduction in <em>M. incognita</em> population (%)</th>
<th>Disease Incidence (%)</th>
<th>Reduction in disease incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>174.14</td>
<td>88.52</td>
<td>23.42</td>
<td>23.84</td>
<td>7.8</td>
<td>17 (24.35)</td>
<td>31.0 (33.83)</td>
<td>36.0 (36.87)</td>
</tr>
<tr>
<td>T2</td>
<td>182.00</td>
<td>104.1</td>
<td>33.08</td>
<td>25.18</td>
<td>6.3</td>
<td>32 (34.45)</td>
<td>28.5 (32.27)</td>
<td>41.0 (39.82)</td>
</tr>
<tr>
<td>T3</td>
<td>192.04</td>
<td>114.6</td>
<td>38.82</td>
<td>35.08</td>
<td>8.2</td>
<td>13 (21.13)</td>
<td>32.8 (34.94)</td>
<td>32.0 (34.54)</td>
</tr>
<tr>
<td>T4</td>
<td>190.00</td>
<td>107.4</td>
<td>35.78</td>
<td>34.8</td>
<td>5.8</td>
<td>38 (38.06)</td>
<td>46.8 (43.17)</td>
<td>3.5 (10.78)</td>
</tr>
<tr>
<td>T5</td>
<td>188.02</td>
<td>104.1</td>
<td>32.42</td>
<td>32.22</td>
<td>7.1</td>
<td>24 (29.33)</td>
<td>44.4 (41.73)</td>
<td>8.4 (16.85)</td>
</tr>
<tr>
<td>T6</td>
<td>205.06</td>
<td>129.5</td>
<td>48.10</td>
<td>30.70</td>
<td>4.9</td>
<td>47 (43.28)</td>
<td>42.6 (40.74)</td>
<td>12.0 (20.27)</td>
</tr>
<tr>
<td>T7</td>
<td>209.30</td>
<td>138.4</td>
<td>45.18</td>
<td>32.86</td>
<td>5.4</td>
<td>42 (40.40)</td>
<td>27.1 (31.37)</td>
<td>44.0 (41.55)</td>
</tr>
<tr>
<td>T8</td>
<td>212.04</td>
<td>150.1</td>
<td>51.66</td>
<td>34.66</td>
<td>4.8</td>
<td>48 (43.85)</td>
<td>33.0 (35.06)</td>
<td>32.0 (34.45)</td>
</tr>
<tr>
<td>T9</td>
<td><strong>220.46</strong></td>
<td><strong>156.04</strong></td>
<td><strong>56.84</strong></td>
<td><strong>34.92</strong></td>
<td><strong>3.0</strong></td>
<td><strong>68 (55.55)</strong></td>
<td><strong>20.1 (26.64)</strong></td>
<td><strong>57.0 (49.02)</strong></td>
</tr>
<tr>
<td>T10</td>
<td>169.20</td>
<td>84.4</td>
<td>28.40</td>
<td>36.66</td>
<td>8.2</td>
<td>13 (21.13)</td>
<td>43.6 (41.32)</td>
<td>10.0 (18.44)</td>
</tr>
<tr>
<td>T11</td>
<td>189.60</td>
<td>106.8</td>
<td>35.40</td>
<td>36.90</td>
<td>6.4</td>
<td>42 (40.40)</td>
<td>45.7 (42.53)</td>
<td>5.0 (12.92)</td>
</tr>
<tr>
<td>T12</td>
<td>168.70</td>
<td>89.0</td>
<td>25.10</td>
<td>28.50</td>
<td>9.4</td>
<td>0 (0.00)</td>
<td>48.5 (44.14)</td>
<td>0</td>
</tr>
<tr>
<td>CD-5%</td>
<td>14.49</td>
<td>7.42</td>
<td>2.35</td>
<td>1.33</td>
<td>0.43</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
Table No. 5.17 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Neem cake on root colonization and yield of okra under field conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root colonization (CFU/g) of <em>P. fluorescens</em> ($x 10^6$)</th>
<th>Root colonization (CFU/g) of <em>P. chlamydosporia</em> ($x 10^5$)</th>
<th>Yield Per plot of 2x2 m (kg)</th>
<th>% increase in yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>3.4</td>
<td>0</td>
<td>15.40</td>
<td>6.94 (15.23)</td>
</tr>
<tr>
<td>T2</td>
<td>5.8</td>
<td>0</td>
<td>16.20</td>
<td>12.5 (20.70)</td>
</tr>
<tr>
<td>T3</td>
<td>6.5</td>
<td>0</td>
<td>16.80</td>
<td>16.66 (24.04)</td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
<td>1.4</td>
<td>15.10</td>
<td>4.86 (12.66)</td>
</tr>
<tr>
<td>T5</td>
<td>0</td>
<td>3.9</td>
<td>15.00</td>
<td>4.16 (11.68)</td>
</tr>
<tr>
<td>T6</td>
<td>0</td>
<td>4.8</td>
<td>16.50</td>
<td>14.58 (22.38)</td>
</tr>
<tr>
<td>T7</td>
<td>3.6</td>
<td>1.8</td>
<td>17.10</td>
<td>18.75 (26.62)</td>
</tr>
<tr>
<td>T8</td>
<td>6.1</td>
<td>4.2</td>
<td>16.50</td>
<td>14.58 (22.38)</td>
</tr>
<tr>
<td>T9</td>
<td><strong>6.7</strong></td>
<td><strong>4.9</strong></td>
<td><strong>18.43</strong></td>
<td><strong>28.00 (31.95)</strong></td>
</tr>
<tr>
<td>T10</td>
<td>0</td>
<td>0</td>
<td>15.8</td>
<td>9.72 (18.15)</td>
</tr>
<tr>
<td>T11</td>
<td>0</td>
<td>0</td>
<td>15.20</td>
<td>5.55 (13.56)</td>
</tr>
<tr>
<td>T12</td>
<td>0</td>
<td>0</td>
<td>14.40</td>
<td>0</td>
</tr>
<tr>
<td>CD-5%</td>
<td>0.75</td>
<td>0.94</td>
<td>1.21</td>
<td>---</td>
</tr>
</tbody>
</table>
Fig No.17 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Neem cake on crop growth of okra under field conditions.

![Effect of combination formulation of *P.chlamydosporia* and *P. fluorescens* enriched Neem Cake on crop growth of okra under field conditions](image)

Fig No.18 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Neem cake on reduction of nematode induced disease complex in okra under field conditions.

![Effect of combination formulation of *P.chlamydosporia* and *P. fluorescens* enriched Neem cake on the reduction of *M. incognita* population and Reduction of Disease incidence under field conditions](image)
Fig No.19 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Neem cake on root colonization and yield of okra under field conditions.

![Diagram showing effect of combination of P. chlamydosporia and P. fluorescens enriched Neem cake on root colonization and yield of okra under field conditions.](image)

Fig No.17 indicates the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Neem cake on crop growth of okra under field conditions. From the figure it is evident that combination of *P. chlamydosporia* and *P. fluorescens* when enriched with Neem cake was very effective in boosting the growth of the okra plant. The treatment No. **T9-PF+PC-SD+SB** was also effective in reducing the nematode induced disease complex in okra under field conditions (Fig No. 18), increasing the root colonization as well as yield of okra under field conditions. (Fig No.19)
Plate No.40 Effect of combination of *P.chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched with Neem cake under field conditions - I

A: Plot where in both okra seeds (SD) and the plot (SB) was treated with combination formulation of *P. chlamydosporia* (PC) and *P. fluorescens* (PF) enriched Neem Cake (NC)

B: Control- untreated plot.
Plate No.41 Effect of combination of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched with Neem cake under field conditions - II

**A:** Plot where in both okra seeds (SD) and the plot (SB) was treated with *P. fluorescens* (PF-7) enriched Neem Cake (NC)

**B:** Control: untreated plot.

**C:** Plot where in both okra seeds (SD) and the plot (SB) was treated with *P. chlamydosporia* (PC-3) enriched Neem Cake (NC)

**D:** Plot where in both okra seeds (SD) and the plot (SB) was treated with combination formulation of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Neem Cake (NC)
Table No.5.18 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with FYM on crop growth of Okra under field conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot Length(cm)</th>
<th>Shoot Wt.(g)</th>
<th>Root Length(cm)</th>
<th>Root Wt.(g)</th>
<th>Root galling index on 1-10 Scale</th>
<th><em>Reduction in M. incognita population (%)</em></th>
<th><em>Disease Incidence (%)</em></th>
<th><em>Reduction in disease incidence (%)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>170.46</td>
<td>83.32</td>
<td>22.4</td>
<td>21.38</td>
<td>7.6</td>
<td>15 (22.79)</td>
<td>31.0 (33.83)</td>
<td>38 (38.06)</td>
</tr>
<tr>
<td>T2</td>
<td>171.48</td>
<td>81.66</td>
<td>23.78</td>
<td>24.34</td>
<td>6.1</td>
<td>30 (33.21)</td>
<td>28.5 (32.27)</td>
<td>43 (40.98)</td>
</tr>
<tr>
<td>T3</td>
<td>184.66</td>
<td>112.3</td>
<td>36.7</td>
<td>33.88</td>
<td>8.3</td>
<td>24 (29.33)</td>
<td>31.8 (34.33)</td>
<td>36 (36.87)</td>
</tr>
<tr>
<td>T4</td>
<td>184.7</td>
<td>104.88</td>
<td>34.24</td>
<td>24.68</td>
<td>5.2</td>
<td>36 (36.87)</td>
<td>45.2 (42.25)</td>
<td>9 (17.46)</td>
</tr>
<tr>
<td>T5</td>
<td>185.26</td>
<td>104.48</td>
<td>28.8</td>
<td>29.4</td>
<td>6.9</td>
<td>27 (31.31)</td>
<td>44.4 (41.78)</td>
<td>11 (19.37)</td>
</tr>
<tr>
<td>T6</td>
<td>198.26</td>
<td>128.38</td>
<td>45.1</td>
<td>27.72</td>
<td>5.9</td>
<td>45 (42.13)</td>
<td>40.3 (39.41)</td>
<td>19 (25.84)</td>
</tr>
<tr>
<td>T7</td>
<td>200.44</td>
<td>151</td>
<td>46.02</td>
<td>26.7</td>
<td>6.4</td>
<td>43 (40.98)</td>
<td>28.1 (32.01)</td>
<td>44 (41.55)</td>
</tr>
<tr>
<td>T8</td>
<td>211.26</td>
<td>150.32</td>
<td>49.88</td>
<td>29.78</td>
<td>5.8</td>
<td>47 (43.28)</td>
<td>32.4 (34.70)</td>
<td>35 (36.27)</td>
</tr>
<tr>
<td>T9</td>
<td>214.6</td>
<td>157.4</td>
<td>52.38</td>
<td>33.26</td>
<td>3.9</td>
<td>57 (49.02)</td>
<td>23.4 (28.93)</td>
<td>53 (46.72)</td>
</tr>
<tr>
<td>T10</td>
<td>161.36</td>
<td>78.26</td>
<td>25.2</td>
<td>23.36</td>
<td>7.2</td>
<td>11 (19.37)</td>
<td>49.8 (44.89)</td>
<td>8 (16.43)</td>
</tr>
<tr>
<td>T11</td>
<td>163.54</td>
<td>82.06</td>
<td>27</td>
<td>21.54</td>
<td>7.1</td>
<td>15 (22.79)</td>
<td>50.0 (45.00)</td>
<td>4 (11.54)</td>
</tr>
<tr>
<td>T12</td>
<td>158.16</td>
<td>76.6</td>
<td>20.94</td>
<td>18.44</td>
<td>8.1</td>
<td>0 (0.00)</td>
<td>50.2 (45.11)</td>
<td>0</td>
</tr>
<tr>
<td>CD-S%</td>
<td>1.71</td>
<td>11.98</td>
<td>2.10</td>
<td>1.82</td>
<td>0.32</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
Table No.5.19 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with FYM on root colonization and yield of okra under open field conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root colonization (CFU/g) of <em>P. fluorescens</em> (x $10^6$)</th>
<th>Root colonization (CFU/g) of <em>P. chlamydosporia</em> (x $10^5$)</th>
<th>Yield Per plot of 2 x 2 m (kg)</th>
<th>* % increase in yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.7</td>
<td>0</td>
<td>12.96</td>
<td>4.51 (12.25)</td>
</tr>
<tr>
<td>T2</td>
<td>4.6</td>
<td>0</td>
<td>12.80</td>
<td>3.22 (10.31)</td>
</tr>
<tr>
<td>T3</td>
<td>5.6</td>
<td>0</td>
<td>13.02</td>
<td>4.76 (12.52)</td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
<td>1.9</td>
<td>13.0</td>
<td>4.83 (12.66)</td>
</tr>
<tr>
<td>T5</td>
<td>0</td>
<td>1.8</td>
<td>13.7</td>
<td>10.48 (18.81)</td>
</tr>
<tr>
<td>T6</td>
<td>0</td>
<td>2.9</td>
<td>13.8</td>
<td>11.29 (19.55)</td>
</tr>
<tr>
<td>T7</td>
<td>3.4</td>
<td>2.1</td>
<td>14.0</td>
<td>12.9 (21.05)</td>
</tr>
<tr>
<td>T8</td>
<td>5.8</td>
<td>3.2</td>
<td>14.5</td>
<td>16.93 (24.27)</td>
</tr>
<tr>
<td>T9</td>
<td>6.2</td>
<td>3.6</td>
<td><strong>14.8</strong></td>
<td><strong>19.35 (26.06)</strong></td>
</tr>
<tr>
<td>T10</td>
<td>0</td>
<td>0</td>
<td>13.40</td>
<td>8.06 (16.43)</td>
</tr>
<tr>
<td>T11</td>
<td>0</td>
<td>0</td>
<td>13.6</td>
<td>9.67 (18.05)</td>
</tr>
<tr>
<td>T12</td>
<td>0</td>
<td>0</td>
<td>12.4</td>
<td>0</td>
</tr>
<tr>
<td>CD-5%</td>
<td>0.59</td>
<td>0.25</td>
<td>1.39</td>
<td>---</td>
</tr>
</tbody>
</table>
Fig No.20 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched FYM on crop growth of okra under field conditions.

![Graph showing the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched FYM on crop growth of okra under field conditions.]

Fig No.21 Effect of combination of *P.chlamydosporia* and *P. fluorescens* enriched FYM on reduction of *M. incognita* population and reduction in disease incidence.

![Graph showing the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched FYM on reduction of *M. incognita* population and Reduction in disease incidence.]

Fig No.22 Effect of combination of *P. chlamydosporia* and *P. Fluorescens* enriched FYM on root colonization and yield of okra under field conditions.

![Graph showing effect of combinations on root colonization and yield](image)

Fig No20 indicates the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched FYM on crop growth of okra under field conditions. From the figure it is evident that combination of *P. chlamydosporia* and *P. fluorescens* when enriched with FYM was very effective in boosting the growth of the okra plant. The treatment no. **T9-PF+PC-SD+SB** was also effective in reducing the nematode induced disease complex in okra under field conditions (Fig No. 21), increasing the root colonization as well as yield of okra under field conditions. (Fig No.22)
Plate No.42 Effect of combination of *P.chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched FYM on crop growth.

**A:** Plot where in both okra seeds (SD) and the plot (SB) was treated with combination formulation of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Farm Yard Manure (FYM)

**B:** Control- untreated plot.
Table No.5.20  Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Vermicompost on crop growth of Okra under field conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot Length</th>
<th>Shoot Wt.(g)</th>
<th>Root Length</th>
<th>Root Wt.(g)</th>
<th>Root galling index on 1-10 Scale</th>
<th>Reduction in <em>M. incognita</em> population (%)</th>
<th>Disease Incidence (%)</th>
<th>Reduction in disease Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>172.5</td>
<td>86</td>
<td>25</td>
<td>24.6</td>
<td>7.4</td>
<td>18 (25.10)</td>
<td>32 (34.45)</td>
<td>30 (33.21)</td>
</tr>
<tr>
<td>T2</td>
<td>173</td>
<td>84.1</td>
<td>30</td>
<td>24.5</td>
<td>6.1</td>
<td>30 (33.21)</td>
<td>28.5 (32.20)</td>
<td>37 (37.47)</td>
</tr>
<tr>
<td>T3</td>
<td>184.3</td>
<td>110</td>
<td>36.7</td>
<td>33.4</td>
<td>7.3</td>
<td>24 (29.33)</td>
<td>31.8 (34.33)</td>
<td>30.5 (33.52)</td>
</tr>
<tr>
<td>T4</td>
<td>186</td>
<td>106</td>
<td>35</td>
<td>31.0</td>
<td>6.0</td>
<td>34 (35.67)</td>
<td>23.4 (28.93)</td>
<td>48 43.85)</td>
</tr>
<tr>
<td>T5</td>
<td>187.2</td>
<td>98</td>
<td>28</td>
<td>26</td>
<td>5.9</td>
<td>32 (34.45)</td>
<td>26.4 (30.92)</td>
<td>42 (40.40)</td>
</tr>
<tr>
<td>T6</td>
<td>185.8</td>
<td>109</td>
<td>46.6</td>
<td>26.5</td>
<td>6.2</td>
<td>30 (33.21)</td>
<td>21.2 (27.42)</td>
<td>53 (46.72)</td>
</tr>
<tr>
<td>T7</td>
<td>204</td>
<td>136.2</td>
<td>43.1</td>
<td>30.1</td>
<td>5.9</td>
<td>38 (38.06)</td>
<td>25.3 (30.20)</td>
<td>44 (41.55)</td>
</tr>
<tr>
<td>T8</td>
<td>208</td>
<td>149.8</td>
<td>48.3</td>
<td>31.4</td>
<td>4.5</td>
<td>45 (42.13)</td>
<td>31.3 (34.02)</td>
<td>31 (33.83)</td>
</tr>
<tr>
<td>T9</td>
<td>212.6</td>
<td>156.4</td>
<td>52.6</td>
<td>32.1</td>
<td>4.1</td>
<td>51 (45.57)</td>
<td>23.1 (28.73)</td>
<td>49 (44.43)</td>
</tr>
<tr>
<td>T10</td>
<td>166.1</td>
<td>80.7</td>
<td>26.4</td>
<td>36.9</td>
<td>7.2</td>
<td>11 (19.37)</td>
<td>39.7 (39.06)</td>
<td>13 (21.13)</td>
</tr>
<tr>
<td>T11</td>
<td>169</td>
<td>100</td>
<td>38.6</td>
<td>34.2</td>
<td>7.6</td>
<td>17 (24.35)</td>
<td>40.1 (39.29)</td>
<td>12 (20.27)</td>
</tr>
<tr>
<td>T12</td>
<td>160</td>
<td>94</td>
<td>25</td>
<td>28.0</td>
<td>8.4</td>
<td>0 (0.00)</td>
<td>45.8 (42.59)</td>
<td>0</td>
</tr>
<tr>
<td>CD-5%</td>
<td>14.87</td>
<td>12.98</td>
<td>1.45</td>
<td>1.91</td>
<td>0.46</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
Table No.5.21 Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Vermicompost on root colonization and yield of okra under field conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root colonization (CFU/g) of <em>P. fluorescens</em> ($x 10^6$)</th>
<th>Root colonization (CFU/g) of <em>P. chlamydosporia</em> ($x 10^5$)</th>
<th>Yield Per plot of 2 x 2 m (kg)</th>
<th><em>% increase in yield</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.1</td>
<td>0</td>
<td>14.03</td>
<td>13.14 (21.22)</td>
</tr>
<tr>
<td>T2</td>
<td>3.8</td>
<td>0</td>
<td>13.89</td>
<td>10.72 (19.09)</td>
</tr>
<tr>
<td>T3</td>
<td>4.8</td>
<td>0</td>
<td>13.92</td>
<td>10.9 (19.28)</td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
<td>1.9</td>
<td>13.5</td>
<td>8.14 (16.54)</td>
</tr>
<tr>
<td>T5</td>
<td>0</td>
<td>0.9</td>
<td>14.6</td>
<td>15.06 (22.79)</td>
</tr>
<tr>
<td>T6</td>
<td>0</td>
<td>1.7</td>
<td>15.4</td>
<td>19.4 (26.13)</td>
</tr>
<tr>
<td>T7</td>
<td>2.7</td>
<td>1.5</td>
<td>16.03</td>
<td>22.6 (28.38)</td>
</tr>
<tr>
<td>T8</td>
<td>4.9</td>
<td>2.8</td>
<td>15.9</td>
<td>22.01 (27.97)</td>
</tr>
<tr>
<td>T9</td>
<td>5.8</td>
<td>2.9</td>
<td><strong>16.89</strong></td>
<td><strong>26.58 (30.98)</strong></td>
</tr>
<tr>
<td>T10</td>
<td>0</td>
<td>0</td>
<td>13.5</td>
<td>8.14 (16.54)</td>
</tr>
<tr>
<td>T11</td>
<td>0</td>
<td>0</td>
<td>14.6</td>
<td>15.06 (22.79)</td>
</tr>
<tr>
<td>T12</td>
<td>0</td>
<td>0</td>
<td>12.4</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>CD-5%</td>
<td>0.47</td>
<td>0.39</td>
<td>1.28</td>
<td>---</td>
</tr>
</tbody>
</table>
**Fig No.23:** Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Vermicompost on crop growth of okra under field conditions.

![Graph showing Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Vermicompost on crop growth of okra in field conditions.](image1)

**Fig No.24:** Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Vermicompost in reduction of *M. incognita* population and reduction in disease incidence under field conditions.

![Graph showing Effect of combination of *P.chlamydosporia* and *P. fluorescens* enriched Vermicompost in reduction of *M. incognita* population and reduction in disease incidence under field conditions.](image2)
Fig No.25: Effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched Vermicompost on root colonization and yield of okra under field conditions

![Graph showing root colonization and yield of okra](image)

Fig No.23 indicates the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched vermicompost on crop growth of okra under field conditions. From the figure it is evident that combination of *P. chlamydosporia* and *P. fluorescens* when enriched with vermicompost was very effective in boosting the growth of the okra plant. The treatment no. **(T9-PF+PC-SD+SB)** was also effective in reducing the nematode induced disease complex in okra under field conditions (Fig No. 24), increasing the root colonization as well as yield of okra under field conditions. (Fig No.25)
Plate No.43 Effect of combination of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Vermicompost on crop growth under field conditions.

A: Plot where in both okra seeds (SD) and the plot (SB) was treated with combination formulation of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Vermicompost (VER)

B: Control- untreated plot.
A: Plot where in both okra seeds (SD) and the plot (SB) was treated with combination formulation of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Neem Cake (NC)

B: Plot where in both okra seeds (SD) and the plot (SB) was treated with combination formulation of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Farm Yard Manure (FYM)

C: Plot where in both okra seeds (SD) and the plot (SB) was treated with combination formulation of *P. chlamydosporia* (PC-3) and *P. fluorescens* (PF-7) enriched Vermicompost (VER)
A: Control plot for combination formulation of *P. chlamydospora* (PC-3) and *P. fluorescens* (PF-7) enriched Neem Cake (NC)

B: Control Plot for combination formulation of *P. chlamydospora* (PC-3) and *P. fluorescens* (PF-7) enriched Vermicompost

C: Control Plot for combination formulation of *P. chlamydospora* (PC-3) and *P. fluorescens* (PF-7) enriched FYM
The field trial photograph was taken to see the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with Neem cake under field conditions (Plate No.40). The crop growth was vigorous and healthy when compared to control.

The field trial photograph taken to see the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched FYM on crop growth under field conditions is shown in Plate No.42.

The field trial photograph taken to see the effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched vermicompost on crop growth under field conditions Plate No.43.

Plate No.44 indicates the all three substrate treatment photos. The effect of combination of *P. chlamydosporia* and *P. fluorescens* enriched with Neem cake, FYM and Vermicompost is shown in this plate and it shows the treatmental differences.