RESULTS

Experiment - I

IN-VITRO EXPERIMENTS

Characteristics of wilt fungus and biocontrol agents

Wilt fungus, *Fusarium udum*

The fungus, *Fusarium udum* was isolated from the diseased plant parts and identified as *Fusarium udum* on the basis of cultural and morphological characters (Fig. 16). The fungus grew up to 50 mm in 5 days on potato dextrose agar (PDA). It produced extensive and cottony mycelium in culture, often with a purple tinge in the mycelium or medium. The mycelium was septate, hyaline and produced three types of spores. Microconidia were small elliptical or with 1-2 septa, whereas the macroconidia were long or curved (fusaroid). Chlamydospores were oval or spherical and formed in older cultures from any cell of the hyphae.

Pathogenicity of *F. udum* was established by proving the Koch’s postulates. The inoculum of *F. udum* prepared on sorghum grains was inoculated in pots containing autoclaved soil. Healthy and surface sterilized seeds were sown and the symptoms of disease were observed 30 days after sowing. The symptoms were identical to those recorded in naturally infested plants. Blackening was sometimes visible through the bark as streaks or bands.

*Trichoderma harzianum*

*T. harzianum* is a fast growing fungus and it covered the entire medium in 90 mm Petri plate in 4 days. It produced septate, hyaline and branched mycelium. The fungus produced spores within 2 days of incubation in alternate concentric rings. Spores were formed on divergent whorls of 2-6 phialides borne on conidiophores. The phialospores were green, sub globose to short ellipsoid 1.5-2.5 μm in size. Chlamydospores were also formed present intercalary in the mycelium. The fully grown PDA plate was light green with fluffy mycelial growth (Fig. 17, 18).
Fig. 16. The pathogen *Fusarium udum*. Colonies from infected tissue (A); vascular browning (B); mycelium (C) and macroconidia (D).
Fig. 17. Colony characters of biocontrol fungi and bacteria.
Fig. 18. Morphological characters of biocontrol fungi and bacteria.
*Trichoderma virens*

The fungus also grew actively but slightly lesser than *T. harzianum* and attained a growth of 70-80 mm in 4 days. Aerial mycelium was floccose initially white and later turned deep green due to sporulation. It produces conidiophores terminated with flask shaped convergent phialides. Conidia from adjacent phialides often coalesced into large gloeoid masses (spore ball) (Fig. 17, 18).

*Pochonia chlamydosporia*

*P. chlamydosporia* is a slow growing fungus and covered 30 mm growth in 10 days on PDA in Petri plates in 10 days. Colonies appeared granular due to the abundant production of dictyochlamydospores which were 15-20 μm in size. The mycelium is white in colour (Fig. 17, 18).

*Bacillus subtilis*

The bacteria produced milky white colonies with rough or sereated margins. The colonies smell earthern. Cells were rod shaped or straight (Fig. 17, 18). It stained violet on performing Gram staining test. The cells in four days old culture possessed endospores. The biochemical and physiological characterization of the strain and soil isolates of *B. subtilis* have been presented in Table 17.

*Pseudomonas fluorescens*

The bacteria produces slimy colonies on the King’s B Agar medium with smooth margins (Fig. 17, 18). It produced fluorescein pigments into the medium. Cells were straight rods and the bacteria stained pink on Gram staining showing its gram -ve nature. The biochemical and physiological characterizations of the strain and soil isolates of *P. fluorescens* have been presented in Table 17.

Comparison of growth rates (monoculture)

*T. harzianum* showed the faster radial growth than *T. virens*. After 72 hrs of inoculation and incubation *T. harzianum* covered the entire surface of PDA plate attaining a growth of 90 mm, whereas *T. virens* attained 81 mm. Rest of the soil isolates also grew luxuriantly but were less efficient than standard strains (Fig. 19).
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* Standard strains otherwise soil isolates; + Tests showing positive response; - Tests showing negative response; NA Not applicable.
Fig. 19. Radial growth of strains/isolates of biocontrol fungi in monoculture in-vitro. Th 00/Tv 00 Standard isolate, Th 01-05/Tv 01-05 Soil isolates.

![Graph showing radial growth of Trichoderma harzianum, T. virens, and Pochonia chlamydosporia.]

Fig. 20. Inhibition of *Fusarium udum* due to strains/isolates of biocontrol agents in-vitro. Th 00/Tv 00/Bs 00/Pf 00 Standard isolate, Others soil isolates.

![Graph showing inhibition of *Fusarium udum*.]
**Dual culture test**

Varied antagonistic potential of *Trichoderma* spp. against *F. udum* was recorded from the periodic observations on the linear growth and colonization of the test pathogen by the antagonist. *T. harzianum* being fast growing gave highest linear growth after 120 hrs. It overgrew on the pathogen mycelium and caused lysis. *T. virens* also overgrew on *F. udum* mycelium but complete lysis of the mycelium did not occurred. Maximum linear growth of *T. virens* was observed after 5 days of incubation. Other soil isolates also inhibited the growth of *F. udum* but were inferior than the standard strains (Fig. 20). The dual culture tests with *P. fluorescens* and *B. subtilis* conducted in the present study have shown 52-67% and 49-62% inhibition in the growth of *F. udum* during 2-6 days, respectively (Fig. 20).

**Mycoparasitism**

*Trichoderma* spp. interacted readily with the mycelium of *F. udum* as was evident by the overgrowth and lysis of mycelium of the pathogenic fungus. Examination of mycelium from the zone of interaction revealed parallel running and coiling of *F. udum* mycelium by the mycelium of *Trichoderma* spp. under microscope. *T. harzianum* was more efficient than *T. virens* and other soil isolates as it completely utilized the *Fusarium* mycelium in 5 days on PDA plates (Fig. 21).

**Effect of volatile compounds**

An inverse relationship, between the age of *Trichoderma* culture and amount of volatile compound production was recorded. Consequently more inhibition in the radial growth of *F. udum* was observed with fresh cultures. Effect of 2, 4, 8 and 10 days old cultures were tested against *F. udum*. Volatile compounds produced by 48 hour old isolates of *Trichoderma* spp. significantly reduced radial mycelial growth of *F. udum*. Maximum inhibition of the mycelial growth of *F. udum* (82%) was recorded by *T. harzianum* followed by *T. virens* (78%). As the cultures of biocontrol agents got older, their effect on the growth of the pathogen significantly decreased (*P*<0.05). The vapour action of volatile compounds from 240 hrs old cultures on the pathogen was minimum (Fig. 22, 24).
Fig. 21. Antifungal activity of *Trichoderma* spp., *Bacillus subtilis* and *Pseudomonas fluorescens* against *F. udum* in dual culture test.
Fig. 22. Inhibition in the colonization by *Fusarium udum* due to volatile compounds produced by *Trichoderma* strains/isolates *in-vitro*.

![Bar chart showing inhibition of *Fusarium udum* colonization by *Trichoderma* strains/isolates.]

Fig. 23. Inhibition in the colonization by *Fusarium udum* due to non-volatile compounds produced by *Trichoderma* strains/isolates *in-vitro*.

![Bar chart showing inhibition of *Fusarium udum* colonization by *Trichoderma* strains/isolates.]

Th 00/Tv 00 Standard isolate, Th 01-05/Tv 01-05 Soil isolate.
Fig. 24. Effect of volatile compounds of *Trichoderma* spp. on growth of *Fusarium udum* (A); phosphate solubilization (B) and antibiotic profiling (C) of biocontrol bacteria.
Effect of non-volatile compounds (culture filtrates)

When *F. udum* was grown on the PDA amended with 10, 30 and 50% concentrations of culture filtrates of biocontrol agents, radial growth of the pathogen was inhibited compared to PDA without culture filtrate (Fig. ). Maximum decrease (78.5%) in the radial growth of *F. udum* was recorded with 50% filtrate of *T. harzianum* filtrate with the concentration of 50% followed by the same concentration of *T. virens* (70%) (Fig. 2, 3).

Nematicidal effects of biocontrol agents *in-vitro*

Nematode hatching and mortality

The hatching of juveniles from egg masses incubated with broth alone (without biocontrol agents) was almost identical to distilled water (Table 18). The hatching was, however, suppressed in culture filtrates. The hatching decreased by 100% in the culture filtrate of *Pochonia chlamydosporia* compared with the control (broth alone) after 5 days of incubation. Treatments with *Pseudomonas fluorescens* (72 - 97%), *Bacillus subtilis* (50 - 84%), *Trichoderma harzianum* (48 - 80%) and *T. virens* (40 - 71%) also inhibited the hatching. The culture filtrates also induced mortality to the juveniles of *M. incognita* (Table 19). Mortality in the juveniles was 65 and 100% with the culture filtrate of *P. chlamydosporia* after 12 and 24 hrs of incubation. Other treatments induced 30 - 65% mortality to the juveniles after 12 - 96 hrs. of incubation. Percent mortality due to *P. fluorescens* filtrate was 55, 76 and 90% after 24, 48 and 96 hrs. of incubation, respectively (Table 19).

Antibiotic profiling of biocontrol bacteria

Antibiotic profiling was done for the used strains of soil bacteria against common 10 antibiotics to develop antibiotic resistant marker strain of *P. fluorescens* and *B. subtilis* (Table 20, 21; Fig. 24). The profiling revealed 25 mg/l medium minimum inhibitory concentration (MIC) of tetracycline hydrochloride (Hi-Media, India) for *P. fluorescens*; from this MIC, minimum tolerance concentration (MTC) of tetracycline i.e., 20 mg/l was determined (Table 21). Resistant marker strain of *P. fluorescens* was developed by subjecting the culture successively from low to high
Table 18. *In vitro* effects of culture filtrate of some soil bacteria on hatching of egg masses of *Meloidogyne incognita* incubated for 5 days.

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<th>Treatments</th>
<th>Distilled water</th>
<th>Broth alone</th>
<th>Culture filtrate</th>
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<tr>
<td></td>
<td></td>
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<td>25%</td>
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<tr>
<td><em>T. virens</em></td>
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<td>119</td>
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<td><em>Pochonia chlamydospora</em></td>
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<td>02</td>
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<td><em>Bacillus subtilis</em></td>
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</tr>
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<td><em>Pseudomonas fluorescens</em></td>
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<td>200</td>
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Values are number of juveniles hatched per egg mass; Each value is mean of three replicates.

Table 19. *In vitro* effects of culture filtrates of some soil bacteria on mortality of *Meloidogyne incognita* juveniles.

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<th>Treatments</th>
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<td>96</td>
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<td><em>T. virens</em></td>
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<td><em>Pochonia chlamydospora</em></td>
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Each value is mean of three replicates.
Table 20. Sensitivity of *Pseudomonas fluorescens* and *Bacillus subtilis* strains/isolates to some common antibiotic drugs.

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Each value is mean of three replicates; Pf *Pseudomonas fluorescens*; Bs *Bacillus subtilis*; R Resistant; * Inhibition zone in mm.

Table 21. Minimum inhibition (MIC) and maximum tolerance (MTC) concentrations (mg/l) of some antibiotic drugs for *Pseudomonas fluorescens* strains/isolates.

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<td>Nitrofurantoin</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>

Each value is mean of three replicates; Pf *Pseudomonas fluorescens*; Bs *Bacillus subtilis*; R Resistant; * Concentration of antibiotics in mg/liter.
concentrations of tetracycline. Tetracycline resistant (20 mg/l) strain of *P. fluorescens* thus obtained was used throughout the study. For the isolation of this bacteria strain from soil, 20 mg tetracycline/l medium was added to Kings B medium to make the medium specific for the tetracycline resistant marker strain of *P. fluorescens*. The MIC could not be determined for *Bacillus subtilis* as the strain was found sensitive to all antibiotics tested.

**Compatibility of fungicides with biocontrol agents**

The fungicide compatibility test of the *Trichoderma* spp. revealed that the maximum growth of the fungi was inhibited (ED$_{90}$) at concentrations 405 and 510 µg carbendazim/ml, 2200 and 2400 µg metalaxyl/ml, 875 and 1000 µg captan/ml, 625 and 755 µg mancozeb/ml and 95 and 150 µg thiram/ml (Table 22). Tolerance for metalaxyl was 5 times higher than carbendazim as 2400 µg/ml concentration of metalaxyl and 500 µg/ml concentration of carbendazim inhibited 90% (ED$_{90}$) growth of *T. harzianum*. The fungicides at concentrations of 60 µg carbendazim/ml, 160 µg captan/ml, 225 µg mancozeb/ml and 1050 µg metalaxyl/ml seem to be safe tolerance limit (ED$_{50}$) for *T. harzianum*. For *T. virens*, ED$_{50}$ 40 µg carbendazim/ml, 125 µg captan/ml, 177 µg mancozeb/ml and 1000 µg metalaxyl/ml. The ED$_{50}$ of thiram for growth of *T. harzianum* and *T. virens* were 150 and 95 µg/ml medium (Table ). Therefore, 25 and 9 µg/ml concentrations seem to be safe tolerance limit (ED$_{50}$) for *T. harzianum* and *T. virens*, respectively. *Pochonia chlamydosporia* showed less tolerance to the five fungicides tested. The fungus was inhibited (ED$_{90}$) by the concentrations of 250 µg carbendazim/ml, 500 µg each of captan and metalaxyl/ml, 350µg mancozeb/ml and 50 µg thiram/ml. Whereas the safe tolerance limit (ED$_{50}$) values were 37.5 µg carbendazim/ml, 75 µg captan/ml, 100 µg metalaxyl/ml, 5 µg thiram/ml and 110 µg mancozeb/ml.

Biocontrol bacteria were found more tolerant to fungicides than fungi (Table 22). The maximum tolerance concentration (MTC) for *B. subtilis* were 3200 µg captan/ml, 60 µg thiram/ml and 600 µg mancozeb/ml. Whereas in case of
Table 22. Fungicide compatibility of *Trichoderma harzianum*, *T. virens*, *Pochonia chlamydosporia*, *Bacillus subtilis* and *Pseudomonas fluorescens* in-vitro.

<table>
<thead>
<tr>
<th></th>
<th><em>T. harzianum</em></th>
<th><em>T. virens</em></th>
<th><em>P. chlamydosporia</em></th>
<th><em>B. subtilis</em></th>
<th><em>P. fluorescens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTC* MIC</td>
<td>MTC MIC</td>
<td>MTC MIC</td>
<td>MTC MIC</td>
<td>MTC MIC</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>60 500</td>
<td>40 405</td>
<td>37.5 250 50,000</td>
<td>-</td>
<td>50,000</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>1050 2400</td>
<td>1000 2200</td>
<td>100 500 7,000 10,000</td>
<td>10,000 25,000</td>
<td></td>
</tr>
<tr>
<td>Captan</td>
<td>160 1000</td>
<td>125 875</td>
<td>75 500 3200 4000</td>
<td>50,000</td>
<td></td>
</tr>
<tr>
<td>Mancozeb</td>
<td>225 755</td>
<td>177 625</td>
<td>110 350 600 1000</td>
<td>1600 2000</td>
<td></td>
</tr>
<tr>
<td>Thiram</td>
<td>25 150</td>
<td>9 95</td>
<td>5 50 60 100</td>
<td>250 3000</td>
<td></td>
</tr>
</tbody>
</table>

* Bach value is mean of three replicates. MTC- Maximum tolerance concentration; MIC- Maximum inhibition concentration. * Concentrations are in µg/ml.

Table 23. Indole acetic acid (IAA) production by *Bacillus subtilis* and *Pseudomonas fluorescens* strains/isolates in liquid medium supplemented with tryptophan 35 mg/100 ml.

<table>
<thead>
<tr>
<th>Organism</th>
<th>IAA (µg/ml)</th>
<th>Organism</th>
<th>IAA (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis (Bs 00)</em></td>
<td>18</td>
<td><em>P. fluorescens (Pf 00)</em></td>
<td>22.6</td>
</tr>
<tr>
<td>Bs 01</td>
<td>10.5</td>
<td>Pf 01</td>
<td>20.5</td>
</tr>
<tr>
<td>Bs 02</td>
<td>13.0</td>
<td>Pf 02</td>
<td>20.0</td>
</tr>
<tr>
<td>Bs 03</td>
<td>9.5</td>
<td>Pf 03</td>
<td>18.0</td>
</tr>
<tr>
<td>Bs 04</td>
<td>16.0</td>
<td>Pf 04</td>
<td>15.0</td>
</tr>
<tr>
<td>Bs 05</td>
<td>13.0</td>
<td>Pf 05</td>
<td>17.0</td>
</tr>
</tbody>
</table>

* Each value is mean of three samples; * Standard strains otherwise soil isolates.

Table 24. Phosphorus solubilization by *Bacillus subtilis* and *Pseudomonas fluorescens* strains/isolates in Pikovskaya’s liquid medium.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Phosphorus (µg/ml)</th>
<th>pH</th>
<th>Bacteria</th>
<th>Phosphorus (µg/ml)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis (Bs)</em></td>
<td>5.6</td>
<td>4.7</td>
<td><em>P. fluorescens (Pf)</em></td>
<td>6.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Bs 01</td>
<td>5.3</td>
<td>5.0</td>
<td>Pf 01</td>
<td>5.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Bs 02</td>
<td>5.4</td>
<td>4.9</td>
<td>Pf 02</td>
<td>5.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Bs 03</td>
<td>5.0</td>
<td>5.2</td>
<td>Pf 03</td>
<td>5.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Bs 04</td>
<td>5.0</td>
<td>5.1</td>
<td>Pf 04</td>
<td>5.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Bs 05</td>
<td>5.3</td>
<td>5.0</td>
<td>Pf 05</td>
<td>5.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>

* Each value is mean of three samples; * Standard strains otherwise soil isolates.
carbendazim the bacteria showed tolerance even for a concentration of 5 g/100 ml (50, 000 μg/ml) (Table ). *P. fluorescens* was found to be more compatible with fungicides than *B. subtilis*, the MTC for the former being 2500 μg Thiram/ml, 1600 μg mancozeb/ml and 5 g/100 ml (50, 000 μg/ml) for captan and carbendazim (Table 22).

**Indole acetic acid (IAA) production in liquid medium**

IAA production varied with isolates. The maximum IAA was produced by the standard strain of *P. fluorescens* (22.6 μg/ml) followed by *B. subtilis* (18.0 μg/ml). The soil isolates produced IAA in the order Pf 1 > Pf 2 > Pf 3 > BS4 (Table 23).

**Phosphate solubilization in liquid medium**

Quantitative estimation of phosphorus solubilization showed maximum solubilization, 6.0 and 5.6 μg P/ml by *P. fluorescens* and *B. subtilis*, respectively followed by 5.8 (Pf 2) and 5.4 μg/ml (BS 2) (Table 24; Fig. 24). The solubilization was coupled with a fall in pH of the medium. The maximum decrease in pH of the medium from 7.0 to 4.6 was recorded with *P. fluorescens* strain/isolates.

**Mass culture of biocontrol agents on agricultural wastes**

Colonization by *T. harzianum* was good (90-100%) on most of the media tested, except husk + sand + molasses (15% colonization) (Table 25; Fig. 25). The spore load of the fungus in terms of colony forming units/g media was greatest on sawdust + molasses and bagasse + soil + molasses. On the husk + sand + molasses, the CFU counts were low. Colonization by *T. virens* was 100% on corn meal + sucrose, bagasse + soil + molasses, wheat meal + sucrose and corn cob + sand + molasses. The CFU count was greatest on sawdust + molasses, corn meal + sucrose mixture,

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*Fig. 25. Biocontrol fungi cultured on various materials in flasks.*
Table 25. Colonization and sporulation of biocontrol fungi, *Trichoderma harzianum*, *T. virens* and *Pochonia chlamydosporia* on agricultural materials.

<table>
<thead>
<tr>
<th>Media</th>
<th><em>T. harzianum</em></th>
<th></th>
<th><em>T. virens</em></th>
<th></th>
<th><em>P. chlamydosporia</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colonization CFU/g %</td>
<td>(x 10^4)</td>
<td>Colonization CFU/g %</td>
<td>(x 10^4)</td>
<td>Colonization CFU/g %</td>
<td>(x 10^4)</td>
</tr>
<tr>
<td>Corn Grain + Sucrose</td>
<td>93</td>
<td>108</td>
<td>80</td>
<td>80</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td>Corn Meal + Sucrose</td>
<td>98</td>
<td>113</td>
<td>100</td>
<td>106</td>
<td>100</td>
<td>110</td>
</tr>
<tr>
<td>Compost + Sucrose</td>
<td>91</td>
<td>102</td>
<td>50</td>
<td>81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leaf litter + Sucrose</td>
<td>100</td>
<td>122</td>
<td>33</td>
<td>17</td>
<td>90</td>
<td>65</td>
</tr>
<tr>
<td>Wheat Grain + Sucrose</td>
<td>100</td>
<td>103</td>
<td>60</td>
<td>84</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Wheat Meal + Sucrose</td>
<td>100</td>
<td>105</td>
<td>100</td>
<td>105</td>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>Oat Kernal + Sucrose</td>
<td>100</td>
<td>108</td>
<td>86</td>
<td>25</td>
<td>50</td>
<td>85</td>
</tr>
<tr>
<td>Corn Cob + Sucrose</td>
<td>90</td>
<td>111</td>
<td>15</td>
<td>24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corn Cob + Molasses</td>
<td>90</td>
<td>113</td>
<td>3</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corn Cob + Sand + Molasses</td>
<td>100</td>
<td>96</td>
<td>100</td>
<td>95</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bagasse + Soil + Molasses</td>
<td>100</td>
<td>124</td>
<td>100</td>
<td>106</td>
<td>90</td>
<td>70</td>
</tr>
<tr>
<td>Saw Dust + Molasses</td>
<td>100</td>
<td>130</td>
<td>100</td>
<td>110</td>
<td>100</td>
<td>78</td>
</tr>
<tr>
<td>Husk + Sand + Molasses</td>
<td>15</td>
<td>2</td>
<td>13</td>
<td>2</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

Each value is mean of three replicates.
bagasse + soil + molasses and wheat meal + sucrose. On husk + sand + molasses and corn cob + molasses, very low CFUs were detected. Colonization by 
P. chlamydosporia\) was 100% on saw dust + molasses, corn meal + sucrose + mixture and wheat meal + sucrose. The CFU counts were greatest on corn meal + sucrose mixture, high but significantly less on oat kernel + sucrose and low on other media. The fungus neither colonized nor produced spores on corn cob + sucrose, corn cob + molasses and corn cob + sand + molasses. Among \textit{Trichoderma} species, \textit{T. harzianum} was the fastest and most efficient colonizer. \textit{P. chlamydosporia} was highly selective, growing on only 8 of the media tested and produced low CFUs. Saw dust + molasses, bagasse + soil + molasses were the best media for mass culture of the biocontrol agents. CFU counts of the organisms on bagasse + soil + molasses and saw dust + molasses were compared with available biopesticides in India. Since India is a major sugarcane producing country, bagasse, saw dust and molasses are available locally at low cost. Their use as a media to mass culture biocontrol fungi will create an opportunity for farmers to produce their own mass culture of organism if a suitable technology is developed and transferred to them.

**Selection of strains/isolates for further study**

Based on \textit{in vitro} performance of the standard strains and soil isolates of biocontrol agents; the standard strains \textit{viz.}, \textit{Trichoderma harzianum} (Th 00), \textit{T. virens} (Tv 00), \textit{Pochonia chlamydosporia} (Pc 00), \textit{Bacillus subtilis} (Bs 00) and \textit{Pseudomonas fluorescens} (Pf 00) were selected for further study for the reason that they showed faster growth rate, had more antipathogenic activity, solubilized phosphorus with greater efficiency and produced more IAA than other isolates.
Experiment II

POT TRIAL ON EVALUATION OF RELATIVE EFFECTIVENESS OF SOME MICROORGANISMS AGAINST WILT, ROOT-KNOT AND WILT DISEASE COMPLEX OF PIGEONPEA

Effects of *Trichoderma harzianum* (Th 00), *T. virens* (Tv 00), *Pochonia chlamydosporia* (Pc 00), *Bacillus subtilis* (Bs 00) and *Pseudomonas fluorescens* (Pf 00) on wilt (*Fusarium udum*), root-knot (*Meloidogyne incognita*) and disease complex (*F. udum + M. incognita*) of pigeonpea were examined in 15 cm diameter clay pots filled with 2 kg sterilized field soil-compost mixture.

**Symptoms**

Fusarial wilt

Plants inoculated with 2 g sorghum seeds colonized by *F. udum* kg soil showed wilt symptoms. The first sign of the disease was mild chlorosis and stunted growth that appeared at seedling stage. Such seedlings usually succumbed due to the infection (Fig. 26). The seedlings which escaped early infection developed chlorosis and

![Fig. 26. Fusarial wilt at seedling stage (A) and preflowering stage (B).](image-url)
stunted growth at one month of age. At a later stage, leaves/branches wilted, drooped and dried (Fig. 27). On average severity of disease was 3.7 on 0-5 scale (Fig. 28). Application of treatments influenced wilt incidence to a varied extent. Seed treatment with *T. harzianum* decreased wilting by 49%, whereas, its soil application resulted to 43% decrease (2.1 on 0-5 scale). Effect of soil application of *Pseudomonas fluorescens* was equal to *T. harzianum* but the seed treatment was slightly less effective (40% decrease). *T. virens* was also equally effective in checking wilt (Fig. 28). Treatments with *Bacillus subtilis* controlled the wilting by 13-16% (*P*<0.05) in comparison to the control. Application of *Pochonia chlamydosporia* did not influence the wilt symptoms. The wilting was checked by 51-54% with the application of carbendazim. The mixture of carbendazim and nemacur decreased the wilting by 30-35%. Nemacur alone, however, did not influence the wilting (Fig. 28).

**Root-knot symptoms**

Plants growing in the plots infested with 2000 J$_2$/kg soil exhibited stunted growth and mild chlorosis in the leaves. On roots specific symptoms in the form of knots were discernibly formed. The galls were, however, small in size (Fig. 27). On average 84 galls and 71 egg masses/root system (Fig. 29) were recorded. The applied treatments suppressed the gall formation and egg mass production but to a varying extent (Fig. 29). Application of *P. chlamydosporia* checked the galling in pigeonpea by 36% (soil application) and 42% (seed treatment) over control. Effect of *P. fluorescens* was almost equal to the *P. chlamydosporia*. Treatments with *B. subtilis*

![Fig. 27. Root galls caused by *Meloidogyne incognita* on pigeonpea.](image-url)
Fig. 28. Effects of seed treatment and soil application of biocontrol agents on the wilt severity (0-5 scale) of pigeonpea caused by *Fusarium udum* alone or with *Meloidogyne incognita*.

![Bar chart showing effects of seed treatment and soil application of biocontrol agents on the wilt severity of pigeonpea.](chart1)

Fig. 29. Effect of seed treatment and soil application of biocontrol agents on the root-knot disease and reproduction of *Meloidogyne incognita* alone or with *Fusarium udum*.

![Bar chart showing effects of seed treatment and soil application of biocontrol agents on root-knot disease and reproduction.](chart2)

Fu- *Fusarium udum*; Mi- *Meloidogyne incognita*
or *Trichoderma* spp. also caused significant decrease in the gall formation. Application of nemacur caused 52-56% decrease in the number of galls/root system. Effect of these treatments on egg mass production was similar to that occurred on gall formation (Fig. 29). Carbendazim alone did not influence the nematode galling or egg mass production but its mixture with nemacur (1:1) resulted to 29-35% and 27-31% decrease in the galls or egg masses, respectively.

### Disease complex

Severity of wilt significantly increased in the pots infested with the *F. udum* and *M. incognita* but galling and egg mass production were significantly decreased in comparison to the pots infested with the respective inoculated plants (Fig. 28, 29). Greatest decrease in the wilt symptoms of concomitantly inoculated plants was recorded due to seed treatment (46%) or soil application (39%) by *P. fluorescens* in comparison to control. *T. harzianum* and *T. virens* were next in effectiveness and decreased the wilting by 32 and 41%, respectively. Application of *B. subtilis* or *P. chlamydosporia* decreased the wilting by 21-27% than the control. In pots applied with mixture of carbendazim and nemacur the wilting was almost 50% less; the pesticides alone, however, checked the wilt symptoms by 38-40 and 5-9%, respectively. Gall formation of concomitantly inoculated plants was reduced by 32 and 39% due to seed and soil treatment with *P. chlamydosporia* compared with the control; corresponding values for *P. fluorescens* were 36 and 24%. With *B. subtilis* treatment the galling was 17-21% less than the control. Soil application or seed treatment with nemacur suppressed the gall formation by 39 and 37%. The mixture of carbendazim and nemacur was, however, less effective than the nematicide alone. The fungicide alone did not influence the galling. More or less similar effects of various treatments were recorded on egg mass production (Fig. 29).

### Dry matter production and yield

Pigeonpea plants applied with *P. fluorescens* produced dry matter and yield 22-27% and 29-35% greater than those not applied with the bacteria (Table 26; Fig. 30). This growth promoting effect was greater with seed treatment. Application of *B. subtilis* also improved the dry matter production of pigeonpea (*P*≤0.05).
Table 26. Effect of seed treatment and soil application of biocontrol agents on the dry matter production and yield of pigeonpea in pots inoculated singly or concomitantly with *Fusarium udum*, *Meloidogyne incognita* or not inoculated.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry shoot weight (g)</th>
<th>Yield/plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed Treatment</td>
<td>Seed Soil Application</td>
</tr>
<tr>
<td>Control (C)</td>
<td>53.5</td>
<td>53.3</td>
</tr>
<tr>
<td><em>T. harzianum</em> (Th)</td>
<td>55.7 (4.2)</td>
<td>54.2 (1.3)</td>
</tr>
<tr>
<td><em>T. virens</em> (Tv)</td>
<td>54.7 (2.2)</td>
<td>54.0 (1.0)</td>
</tr>
<tr>
<td><em>P. chlamydosporia</em> (Pe)</td>
<td>54.1 (1.2)</td>
<td>53.8 (0.6)</td>
</tr>
<tr>
<td><em>B. subtilis</em> (Bs)</td>
<td>63.9* (19.5)</td>
<td>59.5* (11.3)</td>
</tr>
<tr>
<td><em>P. fluorescens</em> (Pf)</td>
<td>68.1* (27.2)</td>
<td>65.4* (22.3)</td>
</tr>
<tr>
<td>Carbendazim (Cb)</td>
<td>55.8 (4.3)</td>
<td>54.8 (2.5)</td>
</tr>
<tr>
<td>Nemacur (Nm)</td>
<td>54.8 (2.5)</td>
<td>54.1 (1.2)</td>
</tr>
<tr>
<td><em>Cb + Nm</em></td>
<td>55.1 (3.0)</td>
<td>54.4 (1.6)</td>
</tr>
<tr>
<td><em>Fusarium</em> (F)</td>
<td>42.0 (-21.5)</td>
<td>42.0 (-21.5)</td>
</tr>
<tr>
<td><em>F + T. harzianum</em> (Th)</td>
<td>50.5* (20.2)</td>
<td>48.8* (16.2)</td>
</tr>
<tr>
<td><em>F + T. virens</em> (Tv)</td>
<td>49.3* (17.4)</td>
<td>47.1* (12.2)</td>
</tr>
<tr>
<td><em>F + P. chlamydosporia</em> (Pe)</td>
<td>43.5 (3.6)</td>
<td>42.5 (1.2)</td>
</tr>
<tr>
<td><em>F + B. subtilis</em> (Bs)</td>
<td>46.8* (11.5)</td>
<td>45.5* (8.3)</td>
</tr>
<tr>
<td><em>F + P. fluorescens</em> (Pf)</td>
<td>58.7* (39.8)</td>
<td>53.1* (26.5)</td>
</tr>
<tr>
<td><em>F + Carbendazim</em> (Cb)</td>
<td>50.2* (19.5)</td>
<td>45.9* (9.4)</td>
</tr>
<tr>
<td><em>F + Nemacur</em> (Nm)</td>
<td>43.1 (2.5)</td>
<td>42.4 (0.9)</td>
</tr>
<tr>
<td><em>F + Cb + Nm</em></td>
<td>43.3* (10.3)</td>
<td>45.5* (8.4)</td>
</tr>
<tr>
<td>Nematode (N)</td>
<td>47.4 (-11.4)</td>
<td>47.4 (-11.4)</td>
</tr>
<tr>
<td><em>N + T. harzianum</em> (Th)</td>
<td>51.9* (9.5)</td>
<td>50.0 (5.4)</td>
</tr>
<tr>
<td><em>N + T. virens</em> (Tv)</td>
<td>51.3* (8.3)</td>
<td>49.4 (4.2)</td>
</tr>
<tr>
<td><em>N + P. chlamydosporia</em> (Pe)</td>
<td>53.3* (12.4)</td>
<td>52.0* (9.8)</td>
</tr>
<tr>
<td><em>N + B. subtilis</em> (Bs)</td>
<td>55.6* (17.4)</td>
<td>52.2* (10.2)</td>
</tr>
<tr>
<td><em>N + P. fluorescens</em> (Pf)</td>
<td>60.4* (27.5)</td>
<td>58.5* (23.4)</td>
</tr>
<tr>
<td><em>N + Carbendazim</em> (Cb)</td>
<td>50.0 (5.4)</td>
<td>49.0 (3.4)</td>
</tr>
</tbody>
</table>

*continued.....*
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (SE)</th>
<th>Mean (SE)</th>
<th>Mean (SE)</th>
<th>Mean (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N + Nemacur (Nm)</td>
<td>53.7 (13.3)</td>
<td>51.8 (9.2)</td>
<td>23.5 (13.3)</td>
<td>22.7 (9.8)</td>
</tr>
<tr>
<td>N + Cb + Nm</td>
<td>51.4 (8.5)</td>
<td>50.9 (7.4)</td>
<td>22.4 (8.3)</td>
<td>22.1 (7.0)</td>
</tr>
<tr>
<td>Nematode + Fusarium (F + N)</td>
<td>26.2 (-51.0)</td>
<td>26.2 (-51.0)</td>
<td>10.6 (-57.0)</td>
<td>10.6 (-57.0)</td>
</tr>
<tr>
<td>F + N + T. harzianum (Th)</td>
<td>31.8 (21.3)</td>
<td>31.0 (18.3)</td>
<td>12.8 (21.2)</td>
<td>12.4 (16.8)</td>
</tr>
<tr>
<td>F + N + T. virens (Tv)</td>
<td>31.3 (19.4)</td>
<td>30.0 (14.4)</td>
<td>12.7 (19.8)</td>
<td>12.0 (13.2)</td>
</tr>
<tr>
<td>F + N + P. chlamydospora Pc</td>
<td>32.6 (24.5)</td>
<td>31.6 (20.5)</td>
<td>12.4 (17.2)</td>
<td>12.1 (14.5)</td>
</tr>
<tr>
<td>F + N + B. subtilis (Bs)</td>
<td>30.2 (15.4)</td>
<td>29.1 (11.2)</td>
<td>13.5 (27.4)</td>
<td>12.9 (21.5)</td>
</tr>
<tr>
<td>F + N + P. fluorescens (Pf)</td>
<td>41.8 (59.4)</td>
<td>37.9 (44.6)</td>
<td>17.2 (62.3)</td>
<td>16.8 (58.4)</td>
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<tr>
<td>F + N + Carbendazim (Cb)</td>
<td>29.1 (11.2)</td>
<td>28.1 (7.3)</td>
<td>12.4 (17.3)</td>
<td>11.8 (11.4)</td>
</tr>
<tr>
<td>F + N + Nemacur (Nm)</td>
<td>30.2 (15.3)</td>
<td>28.8 (9.9)</td>
<td>12.9 (21.5)</td>
<td>12.3 (16.3)</td>
</tr>
<tr>
<td>F + N + Cb + Nm</td>
<td>31.3 (19.6)</td>
<td>29.7 (13.2)</td>
<td>14.3 (35.3)</td>
<td>13.5 (27.4)</td>
</tr>
</tbody>
</table>

L. S. D. (P≤0.05) 3.0 3.5 2.0 2.2

P-value
- Fungus (F) (df=1) 0.000 0.000 0.000 0.000
- Nematode (N) (df=1) 0.000 0.000 0.000 0.000
- Control agents (CA) (df=8) 0.000 0.000 0.000 0.000
- Replicate (df=2) 0.204 0.104 0.098 0.100
- F x N (df=1) 0.000 0.000 0.000 0.000
- F x CA (df=8) 0.000 0.000 0.010 0.205
- N x CA (df=8) 0.000 0.000 0.023 0.550
- F x N x CA (df=8) 0.000 0.165 0.034 0.206

Each value is mean of three replicates; Values in parenthesis are percent increase (+ve) or decrease (-ve) over control; * Significantly different at P≤0.05 otherwise not significant at P≤0.05.
Fig. 30. Effect of seed treatment and soil application of biocontrol agents on the dry matter production and yield of pigeonpea inoculated with *Fusarium udum*, *Meloidogyne incognita* and concomitantly or not inoculated.

<table>
<thead>
<tr>
<th>Fu</th>
<th>Fusarium udum</th>
<th>Mi</th>
<th>Meloidogyne incognita</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fu + Mi</td>
<td><em>F. udum + M. incognita</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>T. harzianum</td>
<td>T. virens</td>
<td></td>
</tr>
<tr>
<td>P. chlamydospora</td>
<td>B. subtilis</td>
<td>P. fluorescens</td>
<td></td>
</tr>
<tr>
<td>Carbendazim (Cb)</td>
<td>Nemat (Nm)</td>
<td>Cb + Nm</td>
<td></td>
</tr>
</tbody>
</table>

**Seed Treatment**

**Soil Application**

*Fu*- *Fusarium udum*; *Mi*- *Meloidogyne incognita*
Infection by the wilt fungus suppressed the dry matter production and yield by 21 and 24%, respectively than the control. Application of various treatments compensated the yield loss but to a varying extent. Seed treatment with \textit{P. fluorescens} promoted ($P \leq 0.05$) the dry matter production and yield by 40 and 30%, respectively. Corresponding values for soil application were 26-27%. \textit{Trichoderma} spp. were next in effectiveness inducing 15-20% enhancement in the variables considered. \textit{B. subtilis} also induced significant effect ($P \leq 0.05$). The plants gave significantly greater yield with the treatments with carbendazim especially through soil application.

Pigeonpea plants infected with root-knot nematode, \textit{M. incognita} produced dry matter and yield 11 and 16% less than the control (Table 26; Fig. 30). Application of \textit{P. fluorescens} significantly promoted the dry matter production and yield of nematode infected plants in comparison to the nematode inoculated control, the effect was 5-6% greater with the seed treatment than soil application. Seed treatment with \textit{B. subtilis} or \textit{P. chlamydosporia} increased the yield by 11%. The yield of pigeonpea was also significantly ($P \leq 0.05$) improved due to application with nemacur.

Concomitant inoculations with wilt fungus and root-knot nematode greatly suppressed the dry matter production (51%) and yield (57%) of pigeonpea over uninoculated control (Table 26, Fig. 30). All treatments applied significantly improved the considered variables of infected plants in comparison to the concomitantly inoculated control. Greatest enhancement in the dry matter and yield of infected plants was recorded with seed treatment (59 and 63%) or soil application (45 and 58%) by \textit{P. fluorescens} compared to the concomitantly inoculated control. Next in effectiveness was \textit{T. harzianum} that gave 21% increase in the yield. Treatment with \textit{B. subtilis}, however, improved the yield by 22-27%. Among pesticide treatments, the mixture of carbendazim and nemacur produced greatest increase in the yield i. e., 27-35% compared to concomitantly inoculated control (Table 26, Fig. 30). ANOVA revealed $P=0.000$ for dry matter for all sources except replicates and fungus x nematode x control agents (soil application) (Table 26). $P$-values for yield were significant ($P \leq 0.05$) for interactions (except fungus x nematode) and replicates under soil application.
**Root nodulation**

The nodulation in pigeonpea was quite good and it further increased due to seed treatment or soil application of *P. fluorescens* and *B. subtilis* being greater with the former (Fig. 31). Infection by *F. udum* and *M. incognita* singly or concomitantly decreased ($P \leq 0.05$) the number of functional and total nodules per root system in comparison to the control (Fig. 32). Decrease in the nodulation by concomitant inoculation of the pathogens was significantly greater than their individual effects. Number of nonfunctional nodules, however, increased ($P \leq 0.05$) in the plants inoculated with the later (Fig. 32).

Application of various treatments checked the suppressive effect of pathogens on root nodulation. Application of *P. fluorescens* through soil or seed treatment increased the number of functional nodules by 44 and 54%, respectively, in the plants infected with the wilt fungus in comparison to the respective controls (Fig. 32). Next in nodule promotion was *B. subtilis, T. harzianum* and carbendazim. These treatments caused significant decrease in the number of nonfunctional nodules.

![Fig. 31. Rhizobial nodules on the roots of pigeonpea. C- control, Fu- nodules colonized by *Fusarium udum* in wilted plants.](image)

Greater increase in the functional nodules of nematode infected plants also occurred due to the application of *P. fluorescens* (32-36%), followed by *B. subtilis* (20-25%) and *P. chlamydosporia* (19-23%) in comparison to nematode inoculated control (Fig. 32). Nemacur application increased the functional nodules by 17-25%, whereas, the mixture of carbendazim and nemacur promoted the
Fig. 32. Effects of seed treatment and soil application of biocontrol agents on the nodulation of pigeonpea inoculated singly or concomitantly with *Fusarium udum* and *Meloidogyne incognita* or not inoculated.

*Fu*- *Fusarium udum*; *Mi*- *Meloidogyne incognita*
nodulation by 8-13% (Fig. 32). Almost similar effect of various treatments was recorded on the nodulation of plants and the order of nodule promotion was *P. fluorescens* > *B. subtilis* > pesticide mixture > *T. harzianum* > *P. chlamydosporia* and *T. virens* (Fig. 32). The order in the decrease of nonfunctional nodules was almost similar to nodule promotion.

**Soil Population**

*Fusarium udum*

Soil population of the wilt fungus, *F. udum* was gradually increased during the four months of crop season from June to October, reaching to a peak of $3.5 \times 10^6$ CFUs/g soil in September in comparison to the planting population of $2.4 \times 10^6$ CFUs (Fig. 33). Various treatments, however, caused decrease in the pathogen population but to a varying extent (Fig. 33). Greatest decrease ($P \leq 0.05$) in the fungus population in comparison to planting population or respective month control was recorded with the seed treatment of carbendazim in July (Fig. 33). Soil or seed treatment with *P. fluorescens* resulted to a decrease of 48-75% in the CFUs of *F. udum*/g soil (Fig. 33). Next in effectiveness was *B. subtilis* through seed treatment and *T. harzianum* through soil application (Fig. 33).

Soil population of *F. udum* was significantly greater ($P \leq 0.05$) in the presence of root-knot nematode, *M. incognita* with an increase of 8-57% in comparison to the monthly population in the absence of nematode (Fig. 33). Population of the fungus in concomitantly inoculated plants was decreased due to application of various treatments in comparison to the respective month control (Fig. 33).

During July, greatest decrease ($P \leq 0.05$) in the population of wilt fungus occurred due to carbendazim. But in later months *P. fluorescens*, *B. subtilis* and *T. harzianum* caused greater or equal decrease in comparison to the planting or respective month control populations (Fig. 33).

From August onwards the greatest decrease ($P \leq 0.05$) in the population of wilt fungus was recorded with *P. fluorescens* followed by *B. subtilis* or *T. harzianum* in comparison to preplant population or respective month control ($P \leq 0.05$).
Fig. 33. Effects of seed treatment and soil application with bioagents on the soil population of *Fusarium udum* in the presence and absence of *Meloidogyne incognita.*
Fig. 34. Effects of seed treatment and soil application with bioagents on the soil population of *Meloidogyne incognita* in the presence and absence of *Fusarium udum*.
**Root-knot nematode**

Soil population of *M. incognita* was gradually increased (*P*≤0.05) with the progress of time from July onwards and reached its peak at harvest that was 116% greater than planting population (Fig. 34). In the presence of wilt fungus, nematode population was significantly (*P*≤0.05) less than the respective month controls.

Seed treatment with nemacur suppressed the July population of nematode by 37% followed by *P. chlamydosporia* 18% (Fig. 34). The August to October populations decreased greater (*P*≤0.05) with the application of *P. chlamydosporia* followed by *B. subtilis*, *P. fluorescens* and nemacur. Mixture of carbendazim and nemacur caused significant decrease (*P*≤0.05) in the population but it was less effective than the nematicide alone (Fig. 34). Soil application of various treatments was relatively less effective than seed treatment in suppressing the nematode population in the absence of wilt fungus (Fig. 34).

In the presence of wilt fungus, nematode population increased (*P*≤0.05) over time, the increase was, however, significantly (*P*≤0.05) less than the nematode alone (Fig. 34). Seed treatment with the biocontrol agents or pesticides decreased (*P*≤0.05) the nematode population. Application of nemacur alone was most effective in decreasing (*P*≤0.05) the nematode population during first month. But in later months, *P. chlamydosporia* caused greater decrease (*P*≤0.05) in the population followed by *B. subtilis*, *P. fluorescens*, nemacur or its combinations with carbendazim (Fig. 34) in comparison to planting or respective month populations. Soil application of various treatments was more or less equally effective in suppressing the nematode population in the presence or absence of wilt fungus.

**Biocontrol agents**

*Trichoderma* species

Soil population of *T. harzianum* increased (*P*≤0.05) during the experimental period, being significant (*P*≤0.05) in August and September (Fig. 35). In soil infested with wilt fungus alone or together with nematode, population of biocontrol agents increased (*P*≤0.05) during all months of sampling being greatest in September in comparison to planting population or respective month control. The increase was
Fig. 35. Rhizosphere population of biocontrol agents in relation to single or concomitant inoculations with *Fusarium udum* and *Meloidogyne incognita*.
relatively greater in pots where nematode was also inoculated. In nematode inoculated pots, increase in *T. harzianum* population was significant in comparison to planting population only. Response of soil population of *T. virens* was more or less similar to *T. harzianum* but the percent increase was relatively less in *T. virens* (Fig. 35).

**Pochonia chlamydosporia**

Soil population of *P. chlamydosporia* applied through seed treatment or soil application in pots not inoculated with either pathogens was marginally increased in comparison to the planting population, the population also decreased in the pots inoculated with wilt fungus (Fig. 35). With the treatments of nematode alone or together with wilt fungus, soil population of *P. chlamydosporia* significantly (*P*≤0.05) increased during all four months in comparison to planting population or respective month control (Fig. 35).

**Bacillus subtilis**

Soil population of *B. subtilis* increased by 10-18% during crop growth from June to October (Fig. 35). Presence of pathogens singly or jointly marginally influenced the population. There was an increase of 10-13% in September and October populations in pots infested with *F. udum* singly or with *M. incognita* in comparison to respective month control (Fig. 35). Response of *B. subtilis* population when applied in soil was almost identical to seed treatment (Fig. 35).

**Pseudomonas fluorescens**

Rhizosphere population of *P. fluorescens* significantly increased during all four months of sampling (Fig. 35) in comparison to planting population. The population of the bacterium further increased (*P*≤0.05) in the presence of pathogens in comparison to respective month controls. Greatest increase in the population was recorded in wilt fungus inoculated pots followed by other treatments of pathogen inoculations in comparison to respective month populations of *P. fluorescens* in the absence of pathogens (Fig. 35). More or less similar population of *P. fluorescens* were recorded where the bacterium was applied to soil but the percent increase was marginally greater in seed treatment (Fig. 35).
Experiment III

FIELD TRIAL TO ASCERTAIN THE EFFECTIVENESS OF SELECTED BIOCONTROL AGENTS AGAINST FUSARIUM WILT, ROOT-KNOT AND WILT DISEASE COMPLEX OF PIGEONPEA

On the basis of performance of five microorganisms tested against *F. udum* and *M. incognita* singly and jointly under pot conditions (Experiment II), *T. harzianum*, *P. chlamydosporia* and *P. fluorescens* were found relatively more effective against the target pathogens, and hence were selected to ascertain their effectiveness under field condition (Fig. 36). Disease suppressing effect of the three microorganisms singly and in combination through soil application and seed treatments was tested in microplots of 4x2 m. Effects of the microorganisms were compared with efficacious pesticides namely carbendazim, nemacur and their combination.

Fig. 36. An aerial view of experimental plots.

Symptoms

Fusarial wilt

The used pigeonpea cultivar (UPAS 120) was found susceptible to the infection by *F. udum* and developed 54% wilt incidence (Fig. 37). Soil application or seed treatment with *T. harzianum* or *P. fluorescens* decreased the wilt incidence by 32-45% and 30-38% being greater with the former (Fig. 38). Greatest decrease in the disease was, however, recorded with the combination of *T. harzianum* and *P. fluorescens* in comparison to the control (Fig. 38). Effect of carbendazim treatment in suppressing the wilt incidence was more or less equal to *T. harzianum*. Application with the mixture of carbendazim and nemacur was 10-12% less effective than carbendazim alone.
Fig. 37. Pigeonpea plants showing wilt symptoms caused by *Fusarium udum*.

**Root-knot**

Plants grown in nematode infested plots showed stunted growth and the foliage was dull green in colour. On underground parts numerous galls were recorded when plants were uprooted at 4 months and roots were examined. The used pigeonpea variety also supported reproduction of the nematode evidenced by 83 egg masses/root system (Fig. 39). Application of various treatments decreased (*P*≤0.05) the nematode symptoms to a varying extent. Seed treatment or soil application with the mixture of *P. chlamydosporia* and *P. fluorescens* resulted to 46% and 27% decrease in the number of galls/root system compared to the control. Next in the effectiveness was individual treatment with both the microorganisms (Fig. 39). However, lowest number of galls were recorded on the plants which were applied with nemacur as soil (58%) or seed treatment (49%) over control. There was more or less proportionate decrease in the egg mass production due to the nematicide application (Fig. 39).

**Disease complex**

Plants grown in the plots inoculated with *F. udum* and *M. incognita* concomitantly exhibited severe stunted growth with chlorotic, wilted and dried leaves. Wilt symptoms in terms of incidence and severity were significantly greater (*P*≤0.05) compared to the wilting recorded in the plots inoculated with *F. udum* alone. Nematode
Fig. 38. Effects of seed treatment and soil application of biocontrol agents on the incidence and severity of wilt of pigeonpea caused by *Fusarium udum* in the presence and absence of *Meloidogyne incognita*.

<table>
<thead>
<tr>
<th>Seed Treatment</th>
<th>Soil Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td><em>Pochonia chlamydosporia (Pc)</em></td>
<td><em>Trichoderma harzianum (Th)</em></td>
</tr>
<tr>
<td><em>Th + Pc</em></td>
<td><em>Pseudomonas fluorescens (Pf)</em></td>
</tr>
<tr>
<td><em>Pc + Pf</em></td>
<td><em>Th + Pf</em></td>
</tr>
<tr>
<td><em>Carbendazim (Cb)</em></td>
<td><em>Th + Pc + Pf</em></td>
</tr>
<tr>
<td><em>Cb + Nm</em></td>
<td><em>Nemacur (Nm)</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wilt incidence (%)</th>
<th>Fu</th>
<th>Fu + Mi</th>
<th>Fu</th>
<th>Fu + Mi</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
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</tr>
<tr>
<td>60</td>
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</tr>
<tr>
<td>50</td>
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</tr>
<tr>
<td>40</td>
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<tr>
<td>30</td>
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<tr>
<td>20</td>
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<td></td>
</tr>
<tr>
<td>10</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wilt severity (0-5 scale)</th>
<th>Fu</th>
<th>Fu + Mi</th>
<th>Fu</th>
<th>Fu + Mi</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
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</tr>
<tr>
<td>4.0</td>
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</tr>
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<td>3.5</td>
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</tr>
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</tr>
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<td>2.5</td>
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<td>2.0</td>
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</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fu- Fusarium udum; Mi- Meloidogyne incognita*
Fig. 39. Effects of seed treatment and soil application of biocontrol agents on the galling caused by *Meloidogyne incognita* in pigeonpea in the presence and absence of *Fusarium udum*.

Fu- *Fusarium udum*; Mi- *Meloidogyne incognita*
symptoms (gall formation and egg mass production), however, significantly decreased ($P<0.05$) in the presence of wilt fungus (Fig. 38, 39). Application of biocontrol agents or pesticides checked the disease symptoms significantly but the greater decrease occurred due to seed treatment or soil application with the combination of *T. harzianum* and/or *P. fluorescens* alone in comparison to concomitantly inoculated control (Fig. 38, 39). Next in effectiveness to check the wilting and galling was carbendazim and nemacur.

**Plant growth and yield**

Treatments with *P. fluorescens* acted as growth promoter to pigeonpea resulting to significant increase in the dry weight of plants and weight of seeds/plant in comparison to inoculated control (Table 27, Fig. 40). Inoculation with *F. udum* suppressed ($P<0.05$) plant dry weight and yield by 32 and 38%, respectively. Application of *P. fluorescens* checked the suppressive effect of the pathogen resulting to significant increase in the dry matter production and yield ($P<0.05$), the enhancement was 20-23% greater with seed treatment (Table 27, Fig. 40). Other treatments such as *T. harzianum* alone or its combination with *P. fluorescens* or all the three biocontrol agents together improved the yield by 27 to 34% over control. The plant growth and yield enhancement due to carbendazim treatments was 23-26% in comparison to inoculated control (Table 27, Fig. 40).

Plants in the plots inoculated with *M. incognita* juveniles produced 20 and 27% less dry matter and yield over control (Table 27, Fig. 40). Seed treatment or soil application with *P. fluorescens* resulted to 32 and 12% increase in the yield of pigeonpea over control. Next in effectiveness was *P. chlamydosporia* with an increase of 11-20% (Table 27, Fig. 40). The combination of *P. fluorescens* and *P. chlamydosporia* promoted the yield by 27% (seed treatment) and 10% (soil application). Soil application of nemacur enhanced the plant growth and yield of pigeonpea by 14 and 22%, respectively over inoculated control; corresponding values for seed treatment were 11 and 18% (Table 27, Fig. 40). Carbendazim application did not provide any significant effect on the dry matter production or yield. But when it was applied along with nemacur, the yield increased significantly ($P<0.05$) but much less than nemacur alone.
Table 27. Effects of seed treatment and soil application of biocontrol agents on the dry matter production and yield of pigeonpea in plots infested singly or concomitantly with *Fusarium udum* and *Meloidogyne incognita* or not inoculated.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry shoot weight (g)</th>
<th>Yield per plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed Treatment</td>
<td>Soil Application</td>
</tr>
<tr>
<td>Control (C)</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td><em>T. harzianum</em> (Th)</td>
<td>89.2 (1.9)</td>
<td>72.8 (-16.8)</td>
</tr>
<tr>
<td><em>P. chlamydospora</em> (Pc)</td>
<td>86.9 (-0.7)</td>
<td>69.3 (-20.8)</td>
</tr>
<tr>
<td><em>P. fluorescens</em> (Pf)</td>
<td>106* (21.1)</td>
<td>98.0* (12.0)</td>
</tr>
<tr>
<td>Th + Pc</td>
<td>88.9 (1.6)</td>
<td>71.4 (-18.4)</td>
</tr>
<tr>
<td>Th + Pf</td>
<td>102* (16.6)</td>
<td>94.0 (7.4)</td>
</tr>
<tr>
<td>Pc + Pf</td>
<td>98.3* (12.3)</td>
<td>94.0 (7.4)</td>
</tr>
<tr>
<td>Th + Pc + Pf</td>
<td>96.3* (10.1)</td>
<td>75.0 (-14.3)</td>
</tr>
<tr>
<td>Carbendazim (Cb)</td>
<td>86.5 (-1.1)</td>
<td>65.4 (-25.3)</td>
</tr>
<tr>
<td>Nemacur (Nm)</td>
<td>85.8 (-1.9)</td>
<td>62.3 (-28.8)</td>
</tr>
<tr>
<td>Cb + Nm</td>
<td>86.2 (-1.5)</td>
<td>69.4 (-20.7)</td>
</tr>
<tr>
<td><em>Fusarium</em> (F)</td>
<td>58.8 (-32.8)</td>
<td>58.8 (-32.8)</td>
</tr>
<tr>
<td><em>T. harzianum</em> + F</td>
<td>74.2* (26.2)</td>
<td>63.5* (8.0)</td>
</tr>
<tr>
<td><em>P. chlamydospora</em> + F</td>
<td>60.6 (3.1)</td>
<td>52.4 (-10.9)</td>
</tr>
<tr>
<td><em>P. fluorescens</em> + F</td>
<td>82.0* (39.4)</td>
<td>69.4* (18.0)</td>
</tr>
<tr>
<td>Th + Pc + F</td>
<td>70.3* (19.6)</td>
<td>62.5 (6.3)</td>
</tr>
<tr>
<td>Th + Pf + F</td>
<td>80.6* (37.1)</td>
<td>65.3* (11.1)</td>
</tr>
<tr>
<td>Pc + Pf + F</td>
<td>68.3* (16.2)</td>
<td>58.4 (-0.7)</td>
</tr>
<tr>
<td>Th + Pc + Pf + F</td>
<td>77.6* (32.0)</td>
<td>63.9* (8.7)</td>
</tr>
<tr>
<td>Carbendazim + F</td>
<td>57.4 (-2.4)</td>
<td>72.7* (23.7)</td>
</tr>
<tr>
<td>Nemacur + F</td>
<td>51.2 (-12.9)</td>
<td>60.1 (2.2)</td>
</tr>
<tr>
<td>Cb + Nm + F</td>
<td>55.9 (-4.9)</td>
<td>68.8* (17.0)</td>
</tr>
<tr>
<td>Nematode (N)</td>
<td>70.4 (-19.5)</td>
<td>70.4 (-19.5)</td>
</tr>
<tr>
<td><em>T. harzianum</em> + N</td>
<td>75.6 (7.3)</td>
<td>73.0 (3.7)</td>
</tr>
<tr>
<td><em>P. chlamydospora</em> + N</td>
<td>81.7* (16.0)</td>
<td>75.9 (7.8)</td>
</tr>
<tr>
<td><em>P. fluorescens</em> + N</td>
<td>90.1* (28.0)</td>
<td>76.5* (8.7)</td>
</tr>
</tbody>
</table>

continued...
<table>
<thead>
<tr>
<th>Treatment</th>
<th>L.S.D. (P&lt;0.05)</th>
<th>4.5</th>
<th>4.4</th>
<th>2.6</th>
<th>3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. S. D. (P&lt;0.05)</td>
<td></td>
<td>4.5</td>
<td>4.4</td>
<td>2.6</td>
<td>3.5</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Fungus (F) (df=1)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Nematode (N) (df=1)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Control agents (CA) (df=10)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Replicate (df=2)</td>
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<td>0.044</td>
<td>0.029</td>
<td>0.053</td>
<td>0.062</td>
</tr>
<tr>
<td>F x N (df=1)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>F x CA (df=10)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>N x CA (df=10)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>F x N x CA (df=10)</td>
<td></td>
<td>0.020</td>
<td>0.000</td>
<td>0.000</td>
<td>0.459</td>
</tr>
</tbody>
</table>

Each value is mean of three replicates; Values in parenthesis are percent increase (+ve) or decrease (-ve) over control; * Significantly different at P<0.05 otherwise not significant at P<0.05.
Fig. 40. Effects of seed treatment and soil application of biocontrol agents on the dry matter production and yield of pigeonpea in plots infested singly or concomitantly with *Fusarium udum* and *Meloidogyne incognita*.

<table>
<thead>
<tr>
<th>Seed Treatment</th>
<th>Soil Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control Fu Mi</td>
</tr>
<tr>
<td><em>Pochonia chlamydosporia (Pc)</em></td>
<td>Fu Mi</td>
</tr>
<tr>
<td><em>Th + Pc</em></td>
<td><em>Th + Pf</em></td>
</tr>
<tr>
<td><em>Pc + Pf</em></td>
<td><em>Th + Pc + Pf</em></td>
</tr>
<tr>
<td><em>Carbendazim (Cb)</em></td>
<td><em>Nemacur (Nm)</em></td>
</tr>
<tr>
<td><em>Cb + Nm</em></td>
<td>Trichoderma harzianum (Th)</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens (Pf)</em></td>
<td>Th Pf</td>
</tr>
</tbody>
</table>

**Fu- *Fusarium udum*; Mi- *Meloidogyne incognita***
Concomitant inoculations with *F. udum* and *M. incognita* caused significantly greater decline in dry matter and yield of pigeonpea in comparison to the sum of reduction caused by the two pathogens individually. Application of *P. fluorescens* singly or in combination of *P. chlamydosporia* greatly increased the yield in comparison to the concomitantly inoculated control. Other combinations of the biocontrol agents improved the yield by 44-51% (seed treatment) and 20-26% (soil application). Among the pesticide treatments, greatest (*P*≤0.05) yield promotion was recorded with the mixture of carbendazim and nemacur through soil application (38%) or seed treatment (29%) in comparison to inoculated control (F/T). Individual effects of the two pesticides were also significant and were in the range of 18-23% and 10-14%, being greater with the nemacur (Table 27, Fig. 40). The *P*-values for all sources were generally 0.000, except for fungus x nemtode x control agents (soil application) and replicates.

**Root nodulation**

Nodulation was also good in the plants grown in microplots under field condition. Nodule formation was further promoted due to application of *P. fluorescens* singly or in combination with other biocontrol agents. Infection by *F. udum* decreased the functional nodule count by 32% whereas, the nonfunctional nodules increased by 22% (Fig. 41). Application of various treatments checked the suppressive effect of wilt fungus on functional nodules leading to a increase in their count. Greatest increase in functional nodules was recorded with seed treatment or soil application with *P. fluorescens* followed by combination of biocontrol agents or *T. harzianum* alone. Carbendazim application also improved the nodulation of fungus inoculated plants.

Root-knot infection also suppressed the nodulation resulting to 29 and 18% decrease in the number of functional and total nodules over control whereas, nonfunctional nodules increased by 17% (Fig. 41). The functional nodules increased in the plants applied with *P. fluorescens* or *P. chlamydosporia* being greater with the former; number of the nonfunctional nodule count was, however, correspondingly decreased especially with the seed treatment. The combination of *P. fluorescens* and *P. chlamydosporia* improved the nodulation 4-9% greater than the *P. fluorescens*
Fig. 41. Effects of seed treatment and soil application of biocontrol agents on the root nodulation in pigeonpea grown in the plots infested singly or concomitantly with *Fusarium udum* and *Meloidogyne incognita.*

<table>
<thead>
<tr>
<th>Seed Treatment</th>
<th>Soil Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Fu</td>
</tr>
<tr>
<td><em>P. chlamydosporia</em> (Pc)</td>
<td>Mi</td>
</tr>
<tr>
<td><em>Th + Pc</em></td>
<td><em>Th + Pf</em></td>
</tr>
<tr>
<td><em>Pc + Pf</em></td>
<td><em>Th + Pc + Pf</em></td>
</tr>
<tr>
<td><em>Carbendazim</em></td>
<td><em>Carbendazim + Nemacur</em></td>
</tr>
</tbody>
</table>

FUNCTIONAL NODULES

NONFUNCTIONAL NODULES

TOTAL NODULES

*Fu-* *Fusarium udum*; *Mi-* *Meloidogyne incognita*
alone (Fig. 41). Effect of nemacur application on promotion in nodulation was more or less equal to *P. fluorescens* alone. Effect of nemacur application on the nodulation was more or less similar to *P. fluorescens* alone (Fig. 41).

Concomitant inoculation with *F. udum* and *M. incognita* greatly decreased the number of functional (63%) and total nodules (33%) in comparison to uninoculated control (Fig. 41). Application of various treatments checked the suppressive effects of the pathogens on root nodulation. Number of functional nodules/root system of infected plants was increased by 79% and 45% due to soil application or seed treatment over uninoculated control (Fig. 41). The nonfunctional nodule count, however, significantly (*P*≤0.05) decreased. Next in effectiveness was combination of *T. harzianum* and *P. fluorescens*, their application on seeds or in soil resulted to 46 and 38% increase in functional nodules, respectively (Fig. 41). Pesticide treatments singly or in combination improved the nodulation by 10-18% in comparison to uninoculated control (Fig. 41).

**Soil population of pathogens and biocontrol agents**

The DNA templates (generated through RAPD-PCR) of 8 or 9 out of 10 randomly picked colonies of the wilt fungus and biocontrol agents recovered through dilution plate method matched with that of their applied strains (Fig. 42). Therefore, it can be concluded that 80-90% of the recovered soil populations of *F. udum*, *T. harzianum*, *T. virens*, *P. chlamydosporia*, *B. subtilis* and *P. fluorescens* were of the strains applied through soil or seed treatment.

**Fusarium udum**

Background population of *F. udum* was very low (<10^2 CfU/g soil) and usually it was below detection level. In the plots where the wilt fungus was applied @ 1.5 g/kg soil, rhizosphere population of *F. udum* was in the range of 10^5 and it further increased during the growth period of four months of the crop reaching its peak in September (Fig. 43). Application of various treatments influenced the soil population of the wilt fungus. Seed treatment with the biocontrol agents was relatively more effective than the soil application in suppressing the population of the
Fig. 42. 1.5% agarose gel showing the RAPD profiles of strains/isolates of the pathogen and biocontrol agents. Lane M: Marker; Lane S: Standard strain; Lanes 1: Recovered soil isolate.
Fig. 43. Effects of seed treatment and soil application with biocontrol agents on the soil population of *Fusarium udum* in the presence and absence of *Meloidogyne incognita*.

![Graph showing effects of seed treatment and soil application with biocontrol agents on the soil population of *Fusarium udum* in the presence and absence of *Meloidogyne incognita*.](image-url)
fungus. This relationship was just reverse for pesticides treatments. Seed treatment with *P. fluorescens* resulted to 46-68% decrease in the CFU of *F. udum* in comparison to respective month controls (Fig. 43). Suppression in the soil population by *T. harzianum* seed treatment was 27-37%. Combination of biocontrol agents especially *T. harzianum* and *P. fluorescens* decreased the CFU count of wilt fungus by 30-46% over control (Fig. 43). Seed treatment with carbendazim resulted to 34-57% decrease in the soil population in comparison to respective month control. Treatment with nemacur did not affect the soil population but, when it was applied with carbendazim a decrease of 28-42% was revealed in the CFUs of wilt fungus in comparison to respective month control (Fig. 43).

In the presence of nematode the soil population of *F. udum* increased further in comparison to *F. udum* alone, the increase was significant (*P*<0.05) at second and third month of sampling. Soil population of *F. udum* in concomitantly inoculated plots was also greatly decreased due to seed treatment (49-58%) and soil application (44-51%) of *P. fluorescens* in comparison to respective month population of concomitantly inoculated control (Fig. 43). Next treatment in effectiveness was the combination of all three biocontrol agents (34-51%) followed by *T. harzianum* + *P. fluorescens* (40-49%). Decrease in the population of wilt fungus due to seed treatment with *T. harzianum* alone was only 20-46% in comparison to respective month control. Carbendazim alone or in combination with nemacur decreased the population by 28-53% in comparison to respective month control (Fig. 43).

**Root-knot nematode**

Soil population of root-knot nematode, *M. incognita* gradually and significantly increased during the crop season of 4 month from July to October in comparison to planting population. Application of *P. chlamydosporia* on seed or in soil decreased the nematode population by 21-51% and 17-43% than the respective month control (Fig. 44). Next in effectiveness was *P. fluorescens*, followed by the combination of *P. chlamydosporia* + *P. fluorescens*. Application of nemacur caused greater decrease in the nematode population during the first two weeks but in later months, its effect was relatively less (Fig. 44). In the plots concomitantly
Fig. 44. Effects of seed treatment and soil application of biocontrol agents on the soil population of *Meloidogyne incognita* in the presence and absence of *Fusarium udum*.

<table>
<thead>
<tr>
<th>Graph</th>
<th>Description</th>
<th>Legend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SEED TREATMENT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nematode</td>
<td>Control ( ), Pseudomonas fluorescens ( ), Th ( ), Pc ( ), Th + Pc ( ), Pc + Pf ( ), Th + Pc + Pf ( ), Nemacur (Nm) ( ), Carbendazim (Cb) ( )</td>
</tr>
<tr>
<td></td>
<td><em>Fusarium + Nematode</em></td>
<td>Th + Pf ( ), Th + Pf ( ), Th + Pf ( ), Th + Pf ( )</td>
</tr>
<tr>
<td><strong>SOIL APPLICATION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nematode</td>
<td>Control ( ), Pseudomonas fluorescens ( ), Th ( ), Pc ( ), Th + Pc ( ), Pc + Pf ( ), Th + Pc + Pf ( ), Nemacur (Nm) ( ), Carbendazim (Cb) ( )</td>
</tr>
<tr>
<td></td>
<td><em>Fusarium + Nematode</em></td>
<td>Th + Pf ( ), Th + Pf ( ), Th + Pf ( ), Th + Pf ( )</td>
</tr>
</tbody>
</table>
inoculated with the two pathogens, nematode population decreased by 8-17% in comparison to respective month population in the plots inoculated with nematode alone (Fig. 44). In these plots greatest decrease in the nematode population occurred due to soil application than seed treatment. The plots which were applied with nemacur singly or with carbenzazim also revealed nematode population significantly less than the respective month controls. Overall effect of biocontrol agents in decreasing nematode population was relatively greater due to seed treatment but for pesticides the effect was reverse (Fig. 44).

**Trichoderma harzianum**

The plots where *T. harzianum* was incorporated through seed or soil application revealed 7-17% greater CFUs of the fungus/g soil compared to planting population during the 4 month growth period of pigeonpea (Fig. 45, 46). The population further increased by 18-30% in the presence of *F. udum* or *F. udum + M. incognita*, being marginally greater in the later (Fig. 45, 46). The plots which received nematode inoculation alone, rarely revealed significant increase in the population of the fungus in comparison to respective month controls. In the combined treatment of *P. chlamydosporia* and *T. harzianum*, the population of the later decreased (*P<0.05*) in comparison to respective month population. In the plots infested with pathogens, the population of *T. harzianum*, however, increased greater in the concomitantly infested plots. In the presence of *P. chlamydosporia*, the soil population of *T. harzianum* marginally decreased during July and August followed by some increase in later two months (Fig. 45, 46). Whereas, the pathogen infested plots revealed significantly (*P≤0.05*) greater CFUs of the antagonist/g soil in comparison to the respective month control. The plots where all three biocontrol agents were applied together, population of *T. harzianum* significantly (*P≤0.05*) decreased during June and July followed by marginal increase in later months, but in the presence of pathogens, the population increased significantly (*P≤0.05*). The increase was, however, less than that recorded in those plots where a combination of two biocontrol agents was applied (Fig. 45, 46). Variation in the *T. harzianum* population where the biocontrol agent(s) was applied through soil application was more or less similar to seed treatment (Fig. 45, 46).
Fig. 45. Soil population of *Trichoderma harzianum* in relation to single and concomitant inoculation with *Fusarium udum* and *Meloidogyne incognita* and seed treatment with biocontrol agents in the rhizosphere of pigeonpea plants grown in microplots.
Fig. 46. Soil population of *Trichoderma harzianum* in relation to single and concomitant inoculation with *Fusarium udum* and *Meloidogyne incognita* and soil application of biocontrol agents in the rhizosphere of pigeonpea plants grown in microplots.

<table>
<thead>
<tr>
<th>Control</th>
<th>Fusarium</th>
<th>Nematode</th>
<th>Fusarium + Nematode</th>
</tr>
</thead>
</table>

**SOIL APPLICATION**

- Colony forming units/g soil (x10^6)
- T. harzianum (Th)
- P. chlamydosporia (Pc) + Th
- P. fluorescens (Pf) + Th
- Th + Pc + Pf

**Pochonia chlamydosporia**

Soil population of *P. chlamydosporia* remained more or less uninfluenced in the plots not incorporated with pathogens during the course of experiment. However, the plots which were inoculated with *M. incognita* singly or concomitantly with *F. udum* revealed significantly (*P*≤0.05) greater population of *P. chlamydosporia* being greater in the former. The population remained unchanged in the presence of *F. udum* alone in comparison to respective month control (Fig. 47, 48).

In the combined treatments with other biocontrol agents, the population of *P. chlamydosporia* remained more or less uninfluenced in comparison to the respective month population of the fungus alone (Fig. 47, 48). In nematode inoculated plots, the population increased significantly, the increase being greater with *P. fluorescens* (Fig. 47, 48). In concomitantly inoculated plots increase in the CFU of *P. chlamydosporia*/g soil was usually significant (*P*≤0.05) in comparison to the respective month population. In the plots which were inoculated with *F. udum* alone, the population of *P. chlamydosporia* did not vary much. Response of soil population of *P. chlamydosporia* in relation to soil application was similar to seed treatment (Fig. 47, 48).

**Pseudomonas fluorescens**

Soil population of *P. fluorescens* remained stagnant during the growth period of pigeonpea (Fig. 49, 50). But in the presence of pathogens it increased significantly (*P*≤0.05). The increase was relatively less in concomitantly inoculated plots. In the combined treatments of biocontrol agents soil population of *P. fluorescens* increased significantly (*P*≤0.05) in the presence of *P. chlamydosporia*. In rest of the combinations the effect was not significant. In the plots infested with the pathogens and applied with combination of biocontrol agents, the significantly (*P*≤0.05) greater CFUs of the bacterium were recorded. This effect was greater in the plots where pathogens were present singly in comparison to respective month controls (Fig. 49, 50). Similarly greater increase in the soil population of *P. fluorescens* was obtained where it was applied with *T. harzianum* or *P. chlamydosporia* in comparison to the combination of three biocontrol agents (Fig. 49, 50).
Fig. 47. Soil population of *Pochonia chlamydospora* in relation to single and concomitant inoculation with *Fusarium udum* and *Meloidogyne incognita* and seed treatment with biocontrol agents in the rhizosphere of pigeonpea plants grown in microplots.
Fig. 48. Soil population of *Pochonia chlamydosporia* in relation to single and concomitant inoculation with *Fusarium udum* and *Meloidogyne incognita* and soil application of biocontrol agents in the rhizosphere of pigeonpea plants grown in microplots.
Fig. 49. Soil population of *Pseudomonas fluorescens* in relation to single and concomitant inoculation with *Fusarium udum* and *Meloidogyne incognita* and seed treatment with biocontrol agents in the rhizosphere of pigeonpea plants grown in microplots.
Fig. 50. Soil population of *Pseudomonas fluorescens* in relation to single and concomitant inoculation with *Fusarium udum* and *Meloidogyne incognita* and soil application of biocontrol agents in the rhizosphere of pigeonpea plants grown in microplots.
Experiment IV

DEVELOPMENT OF BIOPESTICIDES OF TRICHOSTERMA HARZIANUM, POCCHONIA CHLAMYDOSPORIA AND PSEUDOMONAS FLUORESCENS

In view of effectiveness of Trichoderma harzianum, Pochonia chlamydosporia and Pseudomonas fluorescens against wilt, root-knot and disease complex of pigeonpea tested under field condition, their biopesticides were developed.

Production of mass/stock culture of biocontrol agents
Based on relative performance of various agricultural and waste materials viz., seed husk-soil-molasses, sawdust-soil-molasses, bagasse-soil-molasses, leaf litter-molasses, sorghum meal-molasses and sorghum seeds tested for mass production of biocontrol fungi and bacteria, a mixture of sawdust-soil-5% molasses (15:5:1) was selected to grow mass (stock) culture of Trichoderma harzianum, Pochonia chlamydosporia and Pseudomonas fluorescens.

Immobilization of biocontrol agents
After preliminary screening of molasses-lignite-stillage granules, alginate-bran-fermenter biomass pellets, alginate-clay pellets, diatomaceous molasses-soil pellets, sawdust-soil-molasses fermenter biomass, seed husk-sand-molasses fermenter biomass, charcoal powder/pyrex (talc) fermentor biomass powder, fly ash fermenter biomass powder, sodium alginate pellets of liquid fermenter biomass etc., to support survival and multiplication of biocontrol fungi and bacteria, four carriers viz., talc, charcoal, fine clay and fly ash were selected (Fig. 51). The stock culture of biocontrol fungi viz., T. harzianum, P. chlamydosporia and P. fluorescens was mixed in the above mentioned four carriers and 5% molasses in the ratio of 1:0:0, 1:5:1, 1:10:1 (stock culture : carrier : molasses). After 15 days of incubation, the fly ash based formulation revealed highest CFU count in comparison to the other materials used (Fig. 50). The CFU load of T. harzianum, P. chlamydosporia and P. fluorescens increased by 31-117%, 19-40% and 23-71% in fly ash based carrier compared to the stock culture or other carriers, respectively (Fig. 51).
Fig. 51. Multiplication of biocontrol agents in talc, charcoal, fine clay and flyash.
Final Composition of the biopesticides
A mixture of fly ash, soil (loam) and 5% molasses in the ratio of 15:3:1 plus 10 mg chloramphenicol/kg formulation for biocontrol fungi or 45 mg novobiocin, 44.9 mg penicillin and 75 mg cycloheximide/kg formulation for biocontrol bacteria was used as a carrier to immobilize *T. harzianum*, *P. chlamydosporia* and *P. fluorescens*. Thereafter, stock culture was mixed with this carrier in the ratio of 1:5; 1:10; 1:15 and 1:20 and filled in polybags. After 15 days of incubation number of colony forming units (CFUs)/g formulation was determined. The ratio of 20 parts fly ash and one part stock culture was found to be the best in comparison to 1:5; 1:10 and 1:15. The formulations were packed in airtight polypacks of 200, 500 and 1000 g (Fig. 52).

![Fig. 52. Commercial formulation of biopesticides in 200, 500 and 1000 g packets.](image)

Shelf life
The shelf life test of the three biopesticides at five temperature regimes i.e., 5°C, 10°C, 15°C, 25°C and ambient (February to September) for 32 weeks revealed that the biocontrol fungi and bacteria not only remained viable during storage but also multiplied, evidenced by a much greater CFU load during the storage. The CFU load of the biocontrol agents during storage is summarized separately under the following headings supported by Fig. 53.
Biowilt-X (*Trichoderma harzianum*): At ambient temperature, the CFU count of *Trichoderma harzianum* increased significantly in comparison to other temperatures, next was 25°C. Greatest CFU load/g formulation (10^{10}) was recorded during 4 to 12 weeks (Fig. 53). From 12^{th} week onwards, the CFU count gradually declined but even at 16^{th} week of storage it was greater than the control at 25°C or ambient temperature. The fungus was, however, detected in the formulation upto 32 weeks.

Bionem-X (*Pochonia chlamydosporia*): The CFU count of *Pochonia chlamydosporia* in the formulation was greater during 14 weeks (10^9) and reached a peak (3-4 x 10^9) at 10^{th} or 12^{th} week at 25°C or ambient temperature (Fig. 52). Thereafter, the CFU count drastically decreased but still it was at par with the control at 32^{nd} week of storage at 25°C or ambient. At rest of the temperature regimes, the biocontrol fungus was not detected after 14 weeks.

Biocomp-X (*Pseudomonas fluorescens*): The CFU load of *Pseudomonas fluorescens* increased from first week reaching to its peak at 2-12 weeks (9-12 x 10^{13}) at 25°C or ambient temperature (Fig. 52). From 12^{th} week onwards it gradually decreased to a minimum at the 32^{nd} week, but still equal to the control. At rest of the temperatures the CFU load was much low (Fig. 52).
Fig. 53. Shelf life test of the biocontrol agents showing colony forming units per gram formulation at various storage temperatures and durations.
Experiment V

FIELD TRIAL ON EVALUATION OF NEWLY DEVELOPED BIOPESTICIDES FOR EFFECTIVENESS AGAINST FUSARIAL WILT, ROOT-KNOT AND DISEASE COMPLEX OF PIGEONPEA

To evaluate the effectiveness of newly developed biopesticides, Biowilt-X (*Trichoderma harzianum*, Bionem-X (*Pochonia chlamydosporia*) and Biocomp-X (*Pseudomonas fluorescens*) against the target diseases, a field trial was conducted under field condition. The biopesticides were applied to seeds (5 g/kg seed) and soil (40 g/microplot).

SYMPTOMS

Fusarial wilt

Plants grown in the plots infested with *Fusarium udum* developed characteristic symptoms of wilt as described in the previous chapters. Around 55% of the pigeonpea plants growing in fungus inoculated plots showed wilt symptoms with average severity of 3.5 on 0-5 scale (Fig. 54). The wilting was, however, checked due to application of various treatments but to a varying extent. Seed treatment and soil application of *T. harzianum* decreased the wilt incidence by 53 and 32% and the severity by 42 and 29%, respectively over respective control (Fig. 54). The seed treatment with the combination of Biowilt-X and Biocomp-X was more effective than the former alone and checked the wilt severity and incidence by 63 and 50%. Treatment with *P. fluorescens* biopesticide (Biocomp-X) was next in effectiveness and decreased the wilting by 35-44% (seed treatment) and 20-30% (soil application) in comparison to respective controls (Fig. 54). Effect of carbendazim was almost equal to Biowilt-X (*T. harzianum*). Mixture of fungicide and nematicide also checked the wilt symptoms (*P*≤0.05).
Root-knot

Inoculation with 2000 J2 caused characteristic galls on the roots of pigeonpea with an average count of 97 galls and 86 egg masses/root system (Fig. 55). Gall formation and egg mass production decreased significantly ($P \leq 0.05$) due to application of Bionem-X (*P. chlamydosporia*) with marginal difference in seed and soil treatments. Application of Biocomp-X (*P. fluorescens*) also caused similar effects on the nematode disease. The combined treatment of Bionem-X + Biocomp-X was found relatively more effective in suppressing the galling than other combinations. Application of nemacur decreased the galls and egg masses by 40 and 45% (soil application), and 30 and 35% (seed treatment) (Fig. 55). The mixture of nemacur and carbendazim also caused significant ($P \leq 0.05$) decrease in the galling.

Disease complex

The fusarial wilt became more severe on plants which were grown in the plots concomitantly inoculated with *F. udum* and *M. incognita*. Seed treatment with *P. fluorescens* biopesticide or *T. harzianum* biopesticide decreased the wilt incidence by 58 and 40%, and severity by 46 and 36%, respectively over control (Fig. 54). Soil application of the biopesticides was 9-25% less effective than the seed treatment. The combination of Th + Pf biopesticides provided around 50% disease control due to seed treatment and 30% with soil application (Fig. 54). The mixture of carbendazim and nemacur was found more effective than the fungicide alone in checking the wilt symptoms.

The plants grown in concomitantly inoculated plots developed significantly less ($P \leq 0.05$) number of galls and egg masses than those grown in the plots infested with the nematode alone. Application of Bionem-X (*P. chlamydosporia*) or Biocomp-X (*P. fluorescens*) suppressed the galling and egg mass production by 33-35% and 28-35%, respectively over control (Fig. 55). Combination of these two biopesticides was relatively more effective giving a decrease of 38-54% and 33-48% in the galling and egg mass production through seed and soil application, respectively. Among the pesticides greatest decrease in gall and egg mass count occurred due to nemacur alone, 34-46% and 30-39% with soil and seed treatments, respectively (Fig. 55).
Fig. 54. Effects of seed treatment and soil application of newly developed biopesticides on the incidence and severity of wilt caused by *Fusarium udum* in pigeonpea in the presence and absence of *Meloidogyne incognita*.

- **Control**
- **Pochonia chlamydosporia (Pc)**
- **Th + Pc**
- **Pc + Pf**
- **Carbendazim**
- **Carbendazim + Nemacur**
- **Trichoderma harzianum (Th)**
- **Pseudomonas fluorescens (Pf)**
- **Th + Pf**
- **Th + Pc + Pf**
- **Nemacur**

**Fu** - *Fusarium udum*; **Mi** - *Meloidogyne incognita*
Fig. 55. Effects of seed treatment and soil application of newly developed biopesticides on the galling caused by *Meloidogyne incognita* in the presence and absence of *Fusarium udum* in pigeonpea.

Fu- *Fusarium udum*; Mi- *Meloidogyne incognita*
Dry matter production and yield

Application with Biocomp-X (*P. fluorescens*) on seeds or in soil significantly promoted the dry matter production and yield of pigeonpea in comparison to the control (Fig. 55). The yield enhancement was greater due to seed treatment (*P*≤0.05). Other treatments did not provide significant yield enhancement in noninfested plants. Inoculation with *F. udum* caused a suppression of 28 and 37% in the dry matter production and yield of pigeonpea over control (Table 28; Fig. 57). Application of biopesticides or pesticides checked the suppressive effect of pathogens resulting to promotion in the growth and yield of pigeonpea. Seed treatment with Biowilt-X or Biocomp-X enhanced the dry matter production and yield by 28-33% (Table 28; Fig. 57). The combination of the biopesticides also induced significant yield enhancement but it was 7-13% less than Biowilt-X or Biocomp-X treatments. Carbendazim application promoted the yield by 20% (seed treatment) and 28% (soil application) over control. Soil application of pesticide mixture also provided significant (*P*≤0.05) increase in the yield (Table 28; Fig. 57). The *P*-values for all sources were usually 0.000 except fungus x nematode and replicate.

Plants growing in nematode inoculated plots produced 19 and 24% dry matter production and yield less than control (Table 28; Fig. 57). Seed treatment with all the three biopesticides suppressed the pathogenic effect of the nematode and increased the dry matter production and yield in comparison to the control (*P*≤0.05). Greatest yield enhancement occurred with Bionem-X followed by Biocomp-X and Biowilt-X. Among the combinations, the mixture of Biocomp-X and Bionem-X significantly improved the yield. Soil application of nemacur increased the yield and dry matter production by 25-27% in comparison to the respective control (Table 28; Fig. 57). Seed treatment with the nematicide was relatively less effective than the soil application.

Concomitant inoculation with *F. udum* and *M. incognita* caused decrease of 48-59% in the dry matter production and yield of pigeonpea but to a varying extent (Table 28; Fig. 57). Greatest increase in the yield of concomitantly inoculated plants was obtained due to seed application with the combination of Biowilt-X + Biocomp-X (53%). Rest of the combinations of biopesticides increased the yield by 31-38%. Pesticide application improved the yield by 23-32% being greater with the
mixture of carbendazim + nemacur in comparison to the controls (Table 28; Fig. 57). Soil application of biopesticides was 5-13% less effective than the seed treatment whereas, pesticide seed treatment was less effective.

Figure 56. Pigeonpea plants showing wilt symptoms, healthy plants (1); Wilted plants in the absence (2) and presence (3) of root knot nematode; 4. Effect of seed treatment with biocontrol agents on me yield of pigeonpea. A- Control, B- Fusarium udum (Fungus) C- Meloidogyne incognita (Nematode) D- Fungus + Nematode, E- Pseudomonas fluorescens, F- Fungus + Trichoderma harzianum, G- Nematode + P. chlamydosporia + P. fluorescens, H- Fungus + Nematode + T. harzianum + P. fluorescens.

Yield per hectare and cost-benefit ratio

General cultivation: In the control plot, pigeonpea cv. UPAS-120 gave a yield of 18.5 q/ha which is within the prescribed yield of the cultivar used. Application of biopesticides improved the yield, but significant increase was recorded with Biocomp-X (P. fluorescens), its seed treatment resulted to 4.3 q additional yield per hectare worth Rs. 4606/ha after excluding Rs 500/ha as the cost of biopesticide application (Fig. 58).

Fusarial wilt: The wilt disease caused by F. udum inflicted 3.9 q yield loss/ha valuing Rs 4162/ha. The Seed treatment with Biowilt-X (T. harzianum) or
Table 28. Effects of seed treatment and soil application of newly developed biopesticides on the dry matter production and yield of pigeonpea in plots infested singly or concomitantly with *Fusarium udum* and *Meloidogyne incognita* or not infested.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot dry weight (g)</th>
<th>Yield per plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed Treatment</td>
<td>Soil Application</td>
</tr>
<tr>
<td>Control (C)</td>
<td>92.0</td>
<td>92.0</td>
</tr>
<tr>
<td><em>T. harzianum</em> (Th)</td>
<td>96.6 (5.0)</td>
<td>94.9 (3.2)</td>
</tr>
<tr>
<td><em>P. chlamydosporia</em> (Pc)</td>
<td>94.0 (2.5)</td>
<td>92.2 (0.2)</td>
</tr>
<tr>
<td><em>P. fluorescens</em> (Pf)</td>
<td>118 (28.2)</td>
<td>109* (19.0)</td>
</tr>
<tr>
<td>Th + Pc</td>
<td>98.0 (6.6)</td>
<td>94.2 (2.4)</td>
</tr>
<tr>
<td>Th + Pf</td>
<td>99.0 (8.0)</td>
<td>99.0 (7.6)</td>
</tr>
<tr>
<td>Pc + Pf</td>
<td>97.0 (5.6)</td>
<td>95.7 (4.0)</td>
</tr>
<tr>
<td>Th + Pc + Pf</td>
<td>98.0 (7.0)</td>
<td>97.5 (6.0)</td>
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<tr>
<td>Carbendazim (Cb)</td>
<td>92.0 (0.2)</td>
<td>93 (1.0)</td>
</tr>
<tr>
<td>Nemacur (Nm)</td>
<td>90.9 (-1.2)</td>
<td>90.7 (-1.4)</td>
</tr>
<tr>
<td>Cb + Nm</td>
<td>90.6 (-1.5)</td>
<td>93.8 (2.0)</td>
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<td><em>Fusarium</em> (F)</td>
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<td>66.2 (-28.0)</td>
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<tr>
<td><em>T. harzianum</em> + F</td>
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<td>82.2* (24.2)</td>
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<tr>
<td><em>P. chlamydosporia</em> + F</td>
<td>71.0 (7.4)</td>
<td>69.2 (4.5)</td>
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<td><em>P. fluorescens</em> + F</td>
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<td>79.9* (20.7)</td>
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<tr>
<td>Th + Pc + F</td>
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<td>76.2* (15.1)</td>
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<tr>
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<td>77.8* (17.5)</td>
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<td>75.4* (13.9)</td>
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<td>73.8* (11.5)</td>
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<tr>
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<td>78.5 (5.3)</td>
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<td><em>P. chlamydosporia</em> + N</td>
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<tr>
<td><em>P. fluorescens</em> + N</td>
<td>88.7* (19.0)</td>
<td>85.7* (15.0)</td>
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</table>

Continued...
Continued Table 28

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Mean 3</th>
<th>Mean 4</th>
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<td>75.4 (1.2)</td>
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<td>42.9* (25.5)</td>
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<td>86.8* (16.5)</td>
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<tr>
<td>Pc + FN</td>
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<tr>
<td>Pf + FN</td>
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<td>Th + Pc + FN</td>
<td>59.6* (24.6)</td>
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<td>Th + Pf + FN</td>
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<td>Pc + Pf + FN</td>
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<tr>
<td>Th + Pc + Pf + FN</td>
<td>58.8* (23.0)</td>
<td>56.6* (18.4)</td>
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<td>22.7* (23.4)</td>
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<td>22.1* (20.3)</td>
<td>23.6* (28.0)</td>
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<tr>
<td>Cb + Nm + FN</td>
<td>57.8* (21.0)</td>
<td>59.1* (23.5)</td>
<td>23.0* (25.0)</td>
<td>24.4* (32.4)</td>
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L. S. D. (P≤0.05) 5.4 6.9 3.6 3.4

P-value

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<tbody>
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<td>Fungus (F) (df=1)</td>
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<tr>
<td>Nematode (N) (df=1)</td>
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<td>0.000</td>
<td>0.000</td>
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<tr>
<td>Control agents (CA) (df=10)</td>
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</tr>
<tr>
<td>Replicate (df=2)</td>
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<td>0.057</td>
<td>0.091</td>
<td>0.077</td>
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<tr>
<td>F x N (df=1)</td>
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<td>0.000</td>
</tr>
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<td>F x CA (df=10)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>N x CA (df=10)</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
<td>0.038</td>
</tr>
<tr>
<td>F x N x CA (df=10)</td>
<td>0.001</td>
<td>0.004</td>
<td>0.805</td>
<td>0.104</td>
</tr>
</tbody>
</table>

Each value is mean of three replicates; Values in parenthesis are percent increase (+ve) or decrease (-ve) over control; * Significantly different at $P≤0.05$ otherwise not significant at $P≤0.05$. 

Fungus F (df=1) 0.000 0.000 0.000 0.000 
Nematode N (df=1) 0.000 0.000 0.000 0.000 
Control agents CA (df=10) 0.000 0.000 0.000 0.000 
Replicate (df=2) 0.063 0.057 0.091 0.077 
F x N (df=1) 0.000 0.000 0.000 0.000 
F x CA (df=10) 0.000 0.000 0.000 0.000 
N x CA (df=10) 0.000 0.001 0.000 0.038 
F x N x CA (df=10) 0.001 0.004 0.805 0.104
Fig. 57. Effects of seed treatment and soil application of biopesticides on the dry matter production and yield of pigeonpea in plots infested singly or concomitantly with *Fusarium udum* and *Meloidogyne incognita*.

**Seed Treatment**

- Control
- *P. chlamydosporia* (Pc)
- Th + Pc
- Pc + Pf
- Carbendazim (Cb)
- Cb + Nm

**Soil Application**

- T. harzianum (Th)
- *P. fluorescens* (Pf)
- Th + Pf
- Th + Pc + Pf
- Nemacur (Nm)

---

**Shoot dry weight (g)**

**Weight of seeds/ plant (g)**

**Fu- Fusarium udum; Mi- Meloidogyne incognita**
Biocomp-X (*P. fluorescens*) improved the yield of infected pigeonpea crop resulting to a profit of Rs. 3682 and Rs. 6322/ha in comparison to the control (Fig. 58).

**Root-knot nematode:** Root-knot disease caused by *M. incognita* decreased the yield of pigeonpea by 2.8 q/ha costing a monetary loss of Rs 2830/ha (Fig. 58). Seed treatment with Bionem-X (*Pochonia chlamydosporia*) or Biocomp-X (*P. fluorescens*) biopesticides improved the yield equivalent to Rs. 2830-3070/ha.

**Disease complex:** The disease complex caused by *F. udum* and *M. incognita* concomitantly greatly reduced the yield of pigeonpea (7.2 q/ha) valuing Rs 8158/ha (Fig. 58). The Biocomp-X (*P. fluorescens*) was found effective in decreasing the suppressive effect of the pathogens and enhancing the yield. Seed treatment with Biocomp-X gave a profit of Rs. 8998/ha in pigeonpea plots infested with the above two pathogens concomitantly in comparison to the untreated plots (Fig. 58).

**Root nodulation**

On an average 12 nodules/root system were formed. Out of them 9-10 were functional and the remaining nonfunctional (Fig. 59). The nodulation was promoted with the application of Biocomp-X and Biowilt-X singly or jointly being greater with Biocomp-X alone in comparison to the control. Infection by the wilt fungus suppressed the functional nodulation and total nodules by 37 and 25%, respectively, whereas nonfunctional nodules were increased by 16% in comparison to the control. Incorporation of various treatments decreased the suppressive effect of the pathogen on nodulation. Application of Biocomp-X on seed or in soil resulted to 47 and 38% increase in the functional nodules of fungus infected pigeonpea over the infected control. Increase in the functional nodules due to seed treatment with Biowilt-X was 28%, whereas its combination with Biocomp-X gave functional nodules 39% greater than the control. Carbendazim promoted functional nodules by 25% (soil application) and 18% (seed treatment). Suppressive effect of root-knot nematode on nodulation was relatively greater than the wilt fungus resulting to 34 and 23% decrease in the number of functional and total nodules/root system (Fig. 59). Application of Bionem-X or Biocomp-X enhanced the nodulation in pigeonpea with marginally greater effect of the former. Greatest increase in the nodulation,
Figure 58. Yield and cost benefit ratio of seed treatment with biopesticides in pigeonpea (pigeonpea value @ 1200/q; Indian Rs. 100= US $ 2.27).
Fig. 59. Effects of seed treatment and soil application of newly developed biopesticides on the nodulation in pigeonpea in plots infested singly or concomitantly with *Fusarium udum* and *Meloidogyne incognita* or not inoculated.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Seed Treatment</th>
<th>Soil Application</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
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</tr>
<tr>
<td>P. chlamydosporia (Pc)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Th + Pc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pc + Pf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbendazim (Cb)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Th + Pc + Pf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Th + Pf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nemacur (Nm)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FUNCTIONAL NODULES**

**NONFUNCTIONAL NODULES**

**TOTAL NODULES**

*Fu*- *Fusarium udum*; *Mi*- *Meloidogyne incognita*
however, occurred with the combined treatment with Bionem-X + Biocomp-X. Effect of soil application with nemacur was more or less equal to seed treatment with Bionem-X, leading to 27 and 20% increase in the functional and total nodule count.

Concomitant infection by the wilt fungus and root-knot nematode caused 78 and 60% decrease in the functional and total nodules, and an increase in the nonfunctional nodules (Fig. 59). Incorporation of all treatments significantly suppressed the pathogenic effect of wilt fungus and root-knot nematode, resulting to significant increase in the nodulation. Application of Biocomp-X on seed or in soil increased the functional nodules by 51 and 43%, and total nodules by 24 and 16%, respectively. Next in effectiveness was the combination of Biowilt-X and Biocomp-X with or without Bionem-X in promoting functional and total nodules and decreasing nonfunctional nodule count in comparison to the control. Among pesticides soil application of mixture of carbendazim and nemacur was more effective in promoting the functional nodules by 23%. Effect of seed treatment with the biopesticide was relatively more effective than the soil application.

**Soil population of pathogens and biocontrol agents**

The DNA templates (generated through RAPD-PCR) of 8 or 9 out of 10 randomly picked colonies recovered through dilution plate method matched with that of the standard strains of applied pathogen and biocontrol agents (Fig. 42). Hence, it can be said that 80-90% of the recovered populations of *F. udum*, *T. harzianum*, *T. virens*, *P. chlamydosporia*, *B. subtilis* and *P. fluorescens* were of the strains applied through their biopesticides.

**Fusarium udum**

In the plots where *F. udum* was inoculated with out any other treatment, soil population of the wilt fungus increased by 23-44% over the four months from July to October in comparison to planting population (Fig. 60). Different treatments, however, suppressed the population of *F. udum* but to a varying extent. Among the biopesticide treatments lowest population of wilt fungus was recorded in the plots where Biowilt-X was applied through seed (53-73%) or soil (43-65%) application in comparison to respective month control. Treatment with Biocomp-X decreased
Fig. 60. Effects of seed treatment and soil application of neem developed biopesticides on the soil population of *Pseudomonas* in the presence and absence of *Meloidogyne incognita*.
the soil population of the pathogenic fungus by 24-44%. The combined treatments with these two biopesticides resulted to 37-51% (seed treatment) and 28-42% (soil application) over respective controls. Other combinations of biopesticides also induced significant decrease in the population. Carbendazim application, in fact, caused decline in the population of wilt fungus greater than Biowilt-X and the soil application of the fungicide was more effective. Whereas, for biopesticides, the seed treatment caused greater decrease in the population (Fig. 60).

In the plots where root-knot nematode and wilt fungus were inoculated concomitantly, increase in the population of wilt fungus was significantly \( P<0.05 \) greater than in the plots where nematodes were not inoculated (Fig. 60). Seed treatment with Biocomp-X, however, decreased the population of wilt fungus by 51-62% in comparison to the population of concomitantly inoculated control. The combined treatment with Biowilt-X + Biocomp-X was marginally less effective than the later alone and resulted to 35-54% decrease in the soil population of wilt fungus over respective control. Population of \( F. \) udum was also significantly \( P<0.05 \) less in the plots which were applied with other combination of biopesticides. Soil application was less effective than the seed treatment of biopesticides. Effect of fungicide treatment was relatively less effective than Biocomp-X. Application in soil or on seed with the mixture of carbendazim and nemacur resulted to 25-51% and 17-43% decrease in the population of wilt fungus over respective month control (Fig. 60).

**Meloidogyne incognita**

A gradual decrease in the soil population of juveniles of \( M. \) incognita was recorded over the course of experiment (Fig. 61). The nematode population, however, decreased significantly \( P<0.05 \) in the plots where Bionem-X was applied on seeds (29-55%) or in soil (19-48%), in comparison to respective month controls. Application of Biocomp-X singly or in combination with Bionem-X more or less equally suppressed \( P<0.05 \) the nematode population. Other combinations also decreased the population. Soil application or seed treatment with
Fig. 61. Effects of seed treatment and soil application of biopesticides on the soil population of *Meloidogyne incognita* in the presence and absence of *Fusarium udum*.
nemacur decreased the nematode population by 26-39% and 17-26%, respectively. Mixture of carbendazim and nemacur also caused significant decrease in the soil population of *M. incognita*.

In the presence of wilt fungus nematode population decreased by 11-20% in comparison to the respective month control (Fig. 61). Seed treatment with Bionem-X caused greatest decline in the nematode population of concomitantly inoculated plots in comparison to the respective month control. Next in effectiveness was Biocomp-X singly or in combination with Bionem-X. Application of nemacur decreased the nematode population greater than Pc biopesticide. Seed treatment of biopesticide in suppressing the nematode population was relatively more effective than the soil application, for pesticides, soil application was more effective.

**Trichoderma harzianum**

In the plots not inoculated with pathogens but applied with Biowilt-X through soil or seed treatment, soil population of *T. harzianum* increased by 11-20% and 14-22%, respectively in comparison to planting population (Fig. 62, 63). In the presence of pathogens, the population further increased but varied with the pathogen. Greatest increase in the population was recorded in *F. udum* inoculated plots i.e., 17-29% (seed treatment) and 9-20% (soil application) in comparison to respective month controls. In concomitantly inoculated plots, the population was increased by 11-19% and 7-16%. In the combined treatments of Biowilt-X and Biocomp-X, population of *T. harzianum* was significantly greater during September to October over planting population. In rest of the combinations, significant decrease was recorded being greater with Bionem-X.

The fungus infested plots where combination of biopesticides were used revealed significant increase in the soil population of *T. harzianum* being greater with Biowilt-X + Biocomp-X combination in comparison to respective month control (Fig. 62, 63). Similar increase in the population of *T. harzianum* applied through combination was also observed in concomitantly inoculated plots; increase in the population was, however, less than the plots where *F. udum* alone was present. In the plots which were inoculated with root-knot nematode alone, increase in the *T. harzianum* population was rarely significant (*P*≤0.05).
Fig. 62. Soil population of *Trichoderma harzianum* in relation to single and concomitant inoculation with *Fusarium udum* and/or *Meloidogyne incognita* and application of other biopesticides in the rhizosphere of pigeonpea plants grown in microplots.

![Diagram showing the effects of different treatments on soil population of *Trichoderma harzianum* over time.](image-url)
Fig. 63. Soil population of *Trichoderma harzianum* in relation to single and concomitant inoculation with *Fusarium udum* and/or *Meloidogyne incognita* and application of other biopesticides in the rhizosphere of pigeonpea plants grown in microplots.
**Pochonia chlamydosporia**

Soil population of *P. chlamydosporia* in the plots not inoculated with pathogen(s) increased significantly in September to October (Fig. 64, 65). In the plots inoculated with the nematode singly or concomitantly and applied with Bionem-X, the soil population of *P. chlamydosporia* increased further in comparison to respective month control, being greater with nematode alone. In the plots which where inoculated with only *F. udum*, population of *P. chlamydosporia* remained statistically unchanged. In the combined treatments of biopesticides, soil population of *P. chlamydosporia* decreased significantly (*P*≤0.05) except when applied with Biocomp-X in comparison to respective month control. In the presence of pathogens, the population, however, increased. Greatest increase in the population of *P. chlamydosporia* was recorded in the plots inoculated with the combination of Bionem-X + Biocomp-X in comparison to the respective month controls. In *F. udum* inoculated plots population of *P. chlamydosporia* applied in combination with other biopesticides, however, decreased. In general, increase in the *P. chlamydosporia* population was relatively greater while applied on seeds than to the soil (Fig. 64, 65).

**Pseudomonas fluorescens**

Soil population of *P. fluorescens* in uninoculated plots increased by 15-35% (seed treatment) and 17-26% (soil application) in comparison to planting population (Fig. 66, 67). In the plots inoculated with the pathogens singly or concomitantly, population of *P. fluorescens* increased significantly in comparison to respective month control. Increase in the population in the plots inoculated with *F. udum* was greater and than root-knot nematode. In concomitantly inoculated plots, the population increase was more or less equal to the plots which were inoculated with the nematode alone. In the treatments where Biocomp-X was applied in combination with other biopesticide(s) through seed treatment a moderate increase in the soil population of *P. fluorescens* was recorded. In the combination Biowilt-X + Biocomp-X, the increase was significant during all four months of population estimation. Increase in the bacterial population was significant in September and October with
Fig. 64. Soil population of *Pochonia chlamydospora* in relation to single and concomitant inoculation with *Fusarium udum* and *Meloidogyne incognita* and application of other biopesticides in the rhizosphere of pigeonpea plants grown in microplots.
Fig. 65. Soil population of *Pochonia chlamydosporia* in relation to single and concomitant inoculation with *Fusarium udum* and/or *Meloidogyne incognita* and soil application of other biopesticides in the rhizosphere of pigeonpea plants grown in microplots.
Fig. 67. Soil population of *Pseudomonas fluorescens* in relation to single and concomitant inoculation with *Fusarium udum* and/or *Meloidogyne incognita* and soil application of other biopesticides in the rhizosphere of pigeonpea plants grown in microplots.
Fig. 66. Soil population of *Pseudomonas fluorescens* in relation to single and concomitant inoculation with *Fusarium udum* and/or *Meloidogyne incognita* and seed treatment with other biopesticides in the rhizosphere of pigeon pea plants grown in microplots.
Bionem-X and Biocomp-X, and in October with combined application of all three biopesticides. Due to soil application increase in the soil population of \( P. \text{fluorescens} \) was significant only with Biowilt-X + Biocomp-X not in other combinations of biopesticides.

In the presence of pathogens singly or concomitantly, population of \( P. \text{fluorescens} \) increased to a varying extent when combination of biopesticides was applied (Fig. 66, 67). Seed treatment with the Biowilt-X + Biocomp-X in concomitantly inoculated plots resulted to greatest increase in the soil population of \( P. \text{fluorescens} \) (8-21%) in comparison to respective month control. This combination also revealed frequently significant \((P<0.05)\) increase in the population of \( P. \text{fluorescens} \) when applied in fungus or nematode infested plots. With the combination of Bionem-X + Biocomp-X, the population of \( P. \text{fluorescens} \) increased significantly during August and September in concomitantly inoculated plots in comparison to respective month control. Significant increase in the \( P. \text{fluorescens} \) population was also recorded when the combination of all three biopesticides was applied through seed treatment. Increase in the population of \( P. \text{fluorescens} \) was relatively less when the biopesticide(s) was applied to soil (Fig. 66, 67).