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**Research Article**

# Biological control of soil-borne fungal and root-knot nematode disease complex in FCV tobacco nursery

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**ABSTRACT:** Farm yard manure fortified with bio-agents *Pseudomonas fluorescens, Trichoderma asperellum* and *Aspergillus niger* either singly or in rational combinations were evaluated against soil-borne fungal pathogens in FCV tobacco nursery. Results of the trial indicated that application of *P. fluorescens* and *A. niger* fortified FYM @ 4 kg/ $m^2$ recorded 40.2 per cent increase in number healthy transplants (640/ $m^2$) and was on par with recommended chemical schedule (627.5/ $m^2$). At 60 DAS, *P. fluorescens* + *A. niger* fortified FYM, *P. fluorescens* + *T. viride* fortified FYM and chemical check were on par with each other in recording reduced RKI of 1.97, 2.08 and 1.91 respectively compared to 3.80 as RKI in untreated check. Similarly, bio-agents fortified FYM recorded significant decrease in damping off at 35 DAS (41.1 to 52.4%), damping off + blight at 45 DAS (44.1 to 52.9%) and black shank (45.7 to 58.3 %) compared to untreated check. Though, the chemical schedule was superior in decreasing the damping off by 90.0%, damping off + blight by 93.9% and black shank by 93.4% compared to untreated check the study infers the effectiveness of bio-agents for reducing the chemical use to minimize environmental pollution.

**KEY WORDS:** *Aspergillus niger*, FCV tobacco, nursery, *Pseudomonas fluorescens*, soil-borne fungal diseases, *Trichoderma viride*

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## INTRODUCTION

Flue-Cured Virginia tobacco is an important commercial crop grown during *Kharif* in the Southern Transitional Zone of Karnataka from south of Mysore district to Shimoga district. The prevailing weather conditions during nursery phase (March to May) of FCV tobacco influence tremendously the spread of several soil-borne fungal diseases. Damping-off caused by *Pythium aphanidermatum* (Edson) Fitz; *P. myriotylum* (Dreschsler) and *Phytophthora parasitica* var *nicotiane* (Breda de Haan) causing blight and black shank are very important pathogens devastating the nurseries. Humid and warm weather favours the disease epiphytotics. Root-knot incidence due to *Meloidogyne* spp. was major limiting factor in getting healthy and timely transplants (Hussaini, 1983; Shenoi and Nagarajan, 2000). Root-knot infested seedlings transplanted in main field exhibit stunted growth and may even collapse. Losses caused by this nematode are very high when it interacts with other soil-borne-borne fungal pathogens. Several chemical and non-chemical schedules were at present recommended for the control of these diseases (Abdul Wajid *et al*., 1995; Ramakrishnan *et al*., 1998). Indiscriminate use of chemicals results in environmental pollution, residual toxicity and resistance development by the pathogen. Use of bioagents for the control soil-borne fungal pathogens and nematodes has received a greater importance in recent past in many crops (Jayakumar *et al.,* 2007; Ramakrishnan *et al.,* 2009; Seema *et al*., 2011). However, the delivery and time of their application is more important to effectively manage the diseases. The present study was aimed at developing cost effective and eco-friendly, bio-mediated disease management strategy to control soil-borne fungal diseases including root-knot nematodes in FCV tobacco nursery.

## MATERIALS AND METHODS

The studies were conducted at CTRI Research Station, Hunsur during 2009-12 crop season in a replicated trial. The bioagents in commercial formulations @ 1kg were applied to one ton of FYM for fortification. The bioagents *Pseudomonas fluorescens, Trichoderma asperellum* (Sriram *et al*., 2013) and *Aspergillus niger* (AN 27-IARI strain) with load of $10^8$ cfu/g either singly or in combination added to the FYM were incubated for one week with optimum moisture. The heap is turned out at regular intervals to facilitate bioagents multiplication. The treatment combinations were, $T_1$- *P. fluorescens* fortified FYM @ 4kg/$m^2$, $T_2$- *T. asperel-*

*lum* fortified FYM @ 4kg/m$^2$, $T_3$-*A. niger* (AN 27 strain of IARI) fortified FYM @ 4kg/m$^2$, $T_4$- *P. fluorescens* + *T. asperellum* fortified FYM @ 4kg/m$^2$, $T_5$- *P. fluorescens* + *A. niger* fortified FYM @ 4 kg/m$^2$,$T_6$- Recommended chemical check (carbofuran + Ridomil Schedule) and $T_7$- Check. All the seven treatments were replicated four times in randomized block design (RBD) with plot size of one square meter each. The initial mean root-knot nematode population in soil was assessed before treating the bed. The population recorded was 147 second stage juveniles per 100g soil. Beds were watered periodically and followed all other recommended agronomic practices. Germination count was taken at 15 DAS at random in ten squares, each with dimension of 100 sq.cm, from which mean was calculated. Observations on damping off at 30 DAS, blight & black shank diseases at 45 DAS were recorded. Root-knot nematode incidence in terms of Root-knot Index (RKI) was taken on 0-5 scale at 60 DAS. Mean root-knot nematode soil population was also recorded at the time of final transplant pulling. In addition to above, observations on seedling growth parameters such as seedling height (cm) and seedling weight (g) were also, recorded at 60 DAS. Observations on disease free/heathy transplant count were recorded at 60 DAS and at the time of final transplant pulling total healthy transplants count was obtained by adding seedling count in both the pullings. Data collected was subjected to statistical analysis for drawing valid conclusions.

## RESULTS AND DISCUSSION

Results of the trial indicated that, application of antagonistic organisms fortified FYM does not cause any phytotoxicity to tobacco seed germination. At 60 DAS, application of *Pseudomonas fluorescens* + *Aspergillus niger* fortified FYM @ 4 kg/m$^2$ recorded significantly increased number of healthy transplants (640.9 /m$^2$) which was 40.2 per cent increase over check (457/m$^2$). Bio-agents fortified FYM significantly decreased in damping off at 35 DAS (41.1 to 52.4%), damping off + blight at 45 DAS (44.1 to 52.9%) and black shank (45.7 to 58.3 %) compared to untreated check. But the treatments differed significantly from chemical schedule, which was superior in decreasing the damping off by 90%, damping off + blight by 93.9% and black shank by 93.4% compared to untreated check (Table 1 & 2). Such combined application of antagonistic organism may result in synergistic and complementary action against soil-borne fungal pathogens including nematodes in FCV tobacco nursery. Whereas, with regards to root-knot index (RKI) at 60 DAS, antagonistic organism fortified FYM significantly decreased the RKI compared to check. Decline in nematode galling due to application of antagonists fortified FYM ranged from 36.1 to 48.3 per cent over check. Similarly *P. fluorescens*+*A. niger* fortified FYM recorded significantly decreased root-knot nematode population of 85.5$J_2$ per 100 g/soil as compared to 169.4 $J_2$ per 100g/soil at the time of final pulling. Per cent reduction in final soil population effected due to application of bioagent fortified FYM in FCV tobacco beds ranged from 36.7 to 49.5%. Similar to the present results obtained Ramakrishnan and Rajendran (2010) reported application of *P. fluorescens* for effective control of *Meloidogyne arenaria* in groundnut. They had also reported significant increase in plant growth characters and groundnut pod yield in treated plots. Such reduction in nematode disease incidence and soil-population of root-knot nematode and subsequent in-

**Table 1. Effect of antagonists fortified FYM on soil-borne fungal diseases and root-knot nematode in FCV tobacco nursery**

| S. N | Treatment details | Mean germination per100 cm$^2$ (15 DAS) | Damping off at 35DAS | Damping off + Blight At 45DAS | Black shank | RKI at 60 DAS (0-5scale) | Nematode soil population (per100g. Soil) |
|---|---|---|---|---|---|---|---|
| 1. | *P. fluorescens* fortified FYM @4kg/ m$^2$ | 21.8 | 05.62 | 10.75 | 09.62 | 2.47 | 095.5 |
| 2. | *T. viride* fortified FYM @4kg/ m$^2$ | 22.2 | 06.00 | 10.31 | 09.12 | 2.57 | 105.6 |
| 3. | *A. niger* fortified FYM @4kg/ m$^2$ | 20.1 | 06.54 | 10.31 | 11.87 | 2.50 | 107.2 |
| 4. | *P. fluorescens* + *T.viride* fortified FYM @4kg/ m$^2$ | 20.6 | 06.71 | 09.06 | 10.87 | 2.08 | 091.5 |
| 5. | *P. fluorescens* + *A.niger* fortified FYM @4kg/ m$^2$ | 21.3 | 05.42 | 10.25 | 10.62 | 1.97 | 085.5 |
| 6. | Recommended chemical check (Carbofuran + Ridomil MZ schedule) | 21.6 | 01.15 | 01.18 | 01.43 | 1.91 | 081.5 |
| 7. | Untreated check | 20.7 | 11.40 | 19.25 | 21.87 | 3.80 | 169.4 |
| | S.Em | 0.49 | 00.93 | 00.47 | 00.63 | 0.03 | 000.93 |
| | (CD- *P*=0.05) | NS | 02.57 | 01.29 | 01.76 | 0.09 | 002.59 |
| | CV % | 6.50 | 39.67 | 12.98 | 16.66 | 4.79 | 003.08 |

**Table 2. Effect of antagonists fortified FYM on seedling growth and healthy transplants count**

| S.N | Treatment details | Seedling height (cm) | Seedling weight (g) | Healthy trans-plants (60 DAS) | Total healthy transplants count | % increase over check |
|---|---|---|---|---|---|---|
| 1. | *P. fluorescens* fortified FYM @4kg/ $m^2$ | 14.4 | 163.8 | 355.10 | 563.2 | 23.2 |
| 2. | *T. viride* fortified FYM @4kg/ $m^2$ | 14.4 | 164.3 | 343.60 | 542.7 | 18.7 |
| 3. | *A. niger* fortified FYM @4kg/ $m^2$ | 14.5 | 163.3 | 342.50 | 548.4 | 20.0 |
| 4. | *P. fluorescens* + *T. viride* fortified FYM @4kg/ $m^2$ | 14.8 | 169.9 | 372.50 | 586.9 | 28.4 |
| 5. | *P. fluorescens* + *A. niger* fortified FYM @4kg/ $m^2$ | 15.3 | 173.5 | 386.60 | 640.9 | 40.2 |
| 6. | Recommended chemical check (Carbofuran + Ridomil MZ schedule) | 14.4 | 163.3 | 384.30 | 627.5 | 37.3 |
| 7. | Untreated check | 10.1 | 126.5 | 286.30 | 457.0 | - |
| | S.Em | 0.19 | 0.78 | 001.54 | 005.32 | - |
| | (CD- *P*=0.05) | 0.52 | 2.17 | 004.27 | 014.76 | - |
| | CV % | 4.64 | 1.69 | 001.51 | 3.25 | - |

**Table 3. Cost benefit ratio of promising bio-intensive management schedule evolved for FCV tobacco nursery**

| Inputs | Conventional nursery | Bio-intensive management schedule involving *P. fluorescens* + *A.niger* fortified FYM @4kg/ $m^2$ |
|---|---|---|
| Cost of nursery preparation, seed sowing, watering and fertilization ( ₹ ) | 1800/- | 1800/- |
| Cost of Farm Yard Manure (FYM) ( ₹ ) | 500/- | 0500/- |
| Cost of weeding ( ₹ ) | 900/- | 0900/- |
| Cost of bioagent inputs ( ₹ ) | - | 0500/- |
| Total cost ( ₹ ) | 3200/- | 4000/- |
| Additional cost over check ( ₹ ) | | 0800/- |
| Yield of healthy transplants | 45700 | 64100 |
| Number of excess transplants over check | | 18400 |
| Amount realized from transplants (@ ₹ 150 per 1000 transplants) | 6855/- | 9615/- |
| Amount realized from sale of additional transplants ( ₹ ) | - | 2760/- |
| Net returns from the nursery over check ( ₹ ) | - | 2260/- |
| ICBR of the schedule | - | 1:4.5 |

crease in number of root-knot free healthy FCV tobacco transplants due to treatment of nursery beds with *Paecilomyces lilacinus* @ 30g per $m^2$ was obtained by Ramakrishnan and Panduranga Rao (2013). Ramakrishnan and Sreenivas (2012) reported application of *P. fluoroscens* @ 1g/plant in combination with *Aspergillus niger* fortified FYM @ 100g/plant at the time of planting for significant reduction in both Fusarium wilt disease root-knot nematode incidence in FCV tobacco crop. Similar reduction in soil-borne fungal diseases incidence and resultant increase in disease free seedlings for transplantation was reported by Shenoi and Sreenivas (2007).

The study is concluded with identification of a bio-intensive schedule involving application of *P. fluorescens* + *A. niger* fortified FYM @ 4 kg/ $m^2$ as an ideal schedule for the management of soil-borne fungal diseases incited by *Pythium aphanidermatum, Phytophthora parasitica* var *nicotianae* and root-knot nematodes *viz*., *Meloidogyne incognita, M. arenaria* and *M. javanica* in FCV tobacco nurseries with an ICBR of 1:4.5 (Table 3).

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