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Research Article

Evaluation of beneficial fungi in combination with organics against root-knot nematode, *Meloidogyne incognita*, in FCV tobacco nurseries

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ABSTRACT: FCV tobacco is an important commercial crop grown in Karnataka under rain fed situations. Root-knot nematode is a limiting factor causing yield reduction in both nursery and main field crop to the tune of 59.4% and 52.9%, respectively. As an alternative to nematicides of chemical origin, beneficial fungi such as *Paecilomyces lilacinus* and *Glomus fasciculatum* were evaluated along with organic amendments *viz*., neem cake and vermicompost as individual application and also in rational combinations against *Meloidogyne incognita* in FCV tobacco nurseries. Results revealed that application of *P. lilacinus* strain NBAII PLFT5 @ 100g/ m^2 recorded 31.3% increase in healthy transplants compared to check. Combined application of *P. lilacinus* with neem cake @ 1kg/ m^2 recorded 34.4% increase in healthy transplants count and was on par with *P. lilacinus* with vermicompost @ 1kg/m^2. Application of *P. lilacinus* + neem cake @ 1kg/ m^2 and *P. lilacinus* + vermicompost @ 1kg/ m^2 significantly reduced root-knot index to 1.89 compared to 2.05 in carbofuran @ 50g/ m^2 treated beds (standard chemical check) and 3.86 in untreated check. Similarly, these two treatments were on par with each other in significantly reducing the number of egg masses/g root and final soil nematode population.

KEY WORDS: Bio-management, *Glomus fasciculatum*, *Paecilomyces lilacinus*, root-knot nematode, FCV tobacco, neem cake, vermicompost

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INTRODUCTION

Flue Cured Virginia (FCV) tobacco grown under rainfed conditions in Karnataka light soils is the main export oriented commercial crop to several countries earning significant foreign exchange. Root-knot nematode, *Meloidogyne incognita* is a limiting factor causing yield reduction in both nursery and main field crop to the tune of 59.4% and 52.9% respectively (Hussaini, 1983). Root-knot nematode infested seedlings, when transplanted in main field, exhibit stunted growth and may even collapse resulting in lesser plant population. In field, it is observed that the root-knot nematode infection commences in seedlings in the nurseries and spreads to the main field/ new areas through infected planting material. The infected tobacco plants develop a light yellowish cast in contrast to the normal green of healthy plants. Losses caused by this nematode are very high, especially, when they interact with other disease causing pathogens. Although, both fumigant and non-fumigant nematicides such as dazomet and carbofuran have been successfully used against this nematode (Ramakrishnan *et al*., 1998), use of synthetic nematicides in FCV tobacco nursery tends to increase the production cost, besides, causing environmental degradation due to chemical residues in soil and ground water. It is imperative to look for alternative nematode management tactics which are both cost effective and less toxic to environment. Biological suppression of plant parasitic nematodes is gaining importance in view of long-term advantages. Bio-agents like egg parasitic fungus, *Paecilomyces lilacinus* (Samson) Thoms. have been used successfully to manage root-knot nematodes in various crop plants (Ekanayake and Jayasundara, 1994; Jonathan *et al*., 1995). Further, plant health promoting arbuscular fungi (AF) were widely recorded to suppress root-knot nematodes in different plant hosts (Cooper and Grandisons, 1986; Ray and Delei, 1998; Rao and Gowen, 1998; Nagesh *et al.,* 1999). The present communication details the evaluation of these beneficial fungi, *Glomus fasciculatum* (Gerd.) and *P. lilacinus* singly and in combination with organics such as neem cake and vermicompost against root-knot nematode, *M. incognita*, in FCV tobacco nursery.

MATERIALS AND METHODS

The investigation was carried out during 2007-2008 at Central Tobacco Research Institute, Research Station, Hunsur, Karnataka. *P. lilacinus*. strain NBAII PLFT5 isolated locally was formulated as wettable powder with a load of 2 x 10^7 spores/g formulated by NBAII, Bangalore and *G. fasciculatum* was obtained from CTRI, Rajahmundry. Neem cake and vermicompost used in the experiment were locally procured. The biocontrol agents were tested at tobacco nursery as per the treatments given below. The treatment combinations were, T_1 – *G. fasciculatum* (AM) @ 10 g / m^2, T_2- *P. lilacinus* (NBAII strain PL55) @ 100 g/m^2, T_3– carbofuran @ 50 g/m^2, T_4– neem cake @ 1 kg/m^2, T_5- vermicompost @ 1kg/m^2, T_6 – T_1+T4, T_7 – T1+T5, T_8 – T2+T4, T_9 – T2+T5, T_{10} – T1+T2+T4, T_{11} – T1+T2+T5, T_{12} – Check. All the twelve treatments were replicated four times with a mean initial population of 150 second staged juveniles (IJs)/100g of soil. Neem cake and vermicompost were applied a week prior to sowing to facilitate decomposition in raised nursery beds with size of one sq. m., *P. lilacinus* and *G. fasciculatum* were applied at the time of sowing. All the other nursery management practices were followed as per the package of practices of CTRI. Observations on germination count at 10 days after sowing (DAS); number of healthy transplants (total count of transplants free of root-knots) at 60 DAS; Root-Knot Index (RKI) on a 0-5 scale (at 45 and 60 DAS); number of egg masses/g root and final soil nematode population per 100g soil were recorded. Mean seed germination count was obtained by counting the number of seedlings in one m^2, by randomly placing a square (galvanized) iron frame of 100 cm^2 in the nursery beds for five times and calculating the mean at 10 days after sowing. The total number of transplants was obtained by counting the total number of seedlings in two pullings, i.e., one at 60 DAS and at final pulling at 80 DAS. The data were subjected to statistical analysis (CRBD design) using ANOVA and Duncan's multiple range test.

RESULTS AND DISCUSSION

The number of healthy seedlings at 60 DAS for transplantation were significantly higher in all the treated nursery beds compared to untreated (root-knot nematode infested) nursery beds (Table 1). A maximum of 370-370.5 healthy transplants (without nematode infection) per bed

Table 1. Effect of *Paecilomyces lilacinus* and *Glomus fasciculatum* on root-knot free healthy transplants count in FCV tobacco nurseries

Tr. No.	Treatment Details	Mean Germn. Count/100 cm^2	Healthy transplants count at 60 DAS	Total healthy transplants count	Per cent increase over check
T1	*Glomus fasciculatum* (VAM) @ 10 g/m^2	20.7a	292.5b	483.0b	5.9
T2	*Paecilomyces lilacinus* (strain NBAII PLFT5) @ 100 g/m^2	22.1b	352.5	598.1g,h	31.3
T3	Carbofuran@ 50 g/m^2	20.9b	360.0f	611.0i	34.0
T4	Neem Cake@ 1 kg/m^2	21.6a	356.1f	532.5e	16.8
T5	Vermicompost@ 1 kg/m^2	20.5a	313.5c	500.7c	9.9
T6	T1 + T4	21.0b	349.8e	551.2f	20.9
T7	T1 + T5	21.6d	322.5d	524.7d	15.1
T8	T2 + T4	20.9b	370.5h	612.7	34.4
T9	T2 + T5	21.2c	362.5g	614.0i	34.7
T10	T1 + T2 + T4	21.8e	370.0h	613.0i	34.5
T11	T1 + T2 + T5	21.6d	359.0f	595.5g	30.6
T12	Check (Untreated)	21.5d	276.2a	455.7a	–
	S.E.M	0.19	1.77	3.44	–
	CD $P \leq 0.05$	0.33	4.92	9.54	–
	CV %	2.55	1.47	1.75	–

Values followed by different letters are statistically different at $P \leq 0.05$.

were recorded in beds that received a combination of *G. fasciculatum* + *P. lilacinus* + neem cake before sowing, and a combination of *P. lilacinus* + neem cake, which was closely followed by other treated beds, *viz., P. lilacinus* + vermicompost (362.5); carbofuran (360.0); *G. fasciculatum* + *P. lilacinus* + vermicompost (359.0) and neem cake (356.1). Application of neem cake alone and in combinations with the mycorrhiza and/or *P. lilacinus* recorded higher number of healthy transplants of tobacco per m^2 compared to vermicompost and its combinations (Table 1). Among the combinations, *P. lilacinus* with neem cake and or *G. fasciculatum* recorded higher number of healthy transplants per m^2 of nursery bed.

Nematode infection in terms of root-knot index was observed to be significantly the lowest in 60 days-old tobacco seedlings treated with *P. lilacinus* + neem cake (1.30 and 1.89, respectively, at 45 and 60 days after sowing). Both at 45 and 60 days after sowing, root-knot index in treated beds was significantly less compared to the untreated control (Table 2). Per cent decline in tobacco root infection by the nematodes ranged between 29.5 and 51.5 in treated roots over control. Application of *P. lilacinus* in combination with *G. fasciculatum* and neem cake recorded maximum decline in root-knot index (51.5%) over control, which was on par with *P. lilacinus* + neem cake (51.0%), *P. lilacinus* + vermicompost (51.0%), and *P. lilacinus* + *G. fasciculatum* + vermicompost (50.2%) treated seedlings.

Further, number of root-knot nematode egg masses (g /root) were significantly low in tobacco plants treated with carbofuran (11.7), followed by the plants treated with *P. lilacinus* + neem cake (12.1) and on par with *P. lilacinus* + vermicompost (12.2); *P. lilacinus* + *G. fasciculatum* + neem cake (12.4) and *G. fasciculatum* + *P. lilacinus* + vermicompost (12.7) treated plants (Table 2).

It was observed that the root-knot nematode populations in soil (per 100g soil) were reduced to the tune of 19.6 to 53.1% in treated beds compared to the soil in untreated beds (Table 2). A maximum reduction of 53.4% in root-knot nematode populations was recorded in soil treated with *P. lilacinus* + *G. fasciculatum* + neem cake, closely followed by treatments involving *P. lilacinus* + neem cake (53.1%); *P. lilacinus* +

Table 2. Effect of *Paecilomyces lilacinus* and *Glomus fasciculatum* on root-knot disease incidence in FCV tobacco nurseries

Tr.. No.	Treatment Details	Root-knot Index (0 – 5 scale)			No. of egg mass / g. root	Decrease over check (%)	Final soil popln./100g. soil	Decrease over check (%)
		At 45 DAS	At 60 DAS	Decrease over check (%)				
T1	*G. fasciculatum* (AM) @ 10 g/m^2	1.92f	2.72h	29.5	17.4g	13.7	117.5j	19.6
T2	*P. lilacinus* (NBAII strain PLFT5) @ 100 g/m^2	1.50d	2.09d	45.8	13.5d	31.5	75.0e	48.7
T3	Carbofuran@ 50 g/m^2	1.48d	2.05c	46.8	11.7a	40.6	72.5d	50.4
T4	Neem Cake@ 1 kg/m^2	1.78e	2.30e	40.4	15.5e	21.3	83.7f	42.7
T5	Vermicompost@ 1 kg/m^2	1.90f	2.36f	38.8	16.2f	17.7	93.5i	36.0
T6	T1 + T4	1.93f	2.39f	38.1	15.5e	21.3	87.7g	40.0
T7	T1 + T5	1.97g	2.51g	34.9	16.0f	18.8	91.1h	37.6
T8	T2 + T4	1.30a	1.89a	51.0	12.1b	38.5	68.7a	53.1
T9	T2 + T5	1.39b	1.89a	51.0	12.2b	38.1	69.2a,b	52.6
T10	T1 + T2 + T4	1.37b	1.87a	51.5	12.4a,b	37.1	68.2a	53.4
T11	T1 + T2 + T5	1.42b,c	1.92a,b	50.2	12.7c	35.5	70.0c	52.1
T12	Check (Untreated)	2.54h	3.86i	–	19.7h	–	146.2k	–
	SEm	0.04	0.03		0.28		0.71	
	CD $P \leq 0.05$	0.11	0.08		0.77		1.96	
	CV %	6.80	3.52		5.38		2.30	

Values followed by different letters are statistically different at $P \leq 0.05$.

vermicompost (52.6%) and *P. lilacinus* + *G. fasciculatum* + vermicompost (52.1%).

Earlier, Bagyaraj *et al*. (1979) tested the effects of *Glomus mosseae* and *Paecilomyces lilacinus* on *Meloidogyne javanica* of tomato in a greenhouse experiment and observed that the highest root development was achieved when mycorrhizal plants were inoculated with *P. lilacinus*. Inoculation of tomato plants with *G. mosseae* suppressed gall index and the average number of galls per root system by 52% and 66%, respectively, compared to the seedlings inoculated with *M. javanica* alone. Biological control with both *G. mosseae* and *P. lilacinus,* together or separately, in the presence of layer manure completely inhibited root infection with *M. javanica*. Mycorrhizal colonization was not affected by the layer manure treatment or by root inoculation with *P. lilacinus*. Addition of layer manure had a beneficial effect on plant growth and reduced *M. javanica* infection.

In a similar study, Nagesh and Singh (2004), evaluated bio-priming or seed dressing of planting material in tuberose with a combination of nematode antagonist, *Pochonia chlamydosporia* (5 x 10^3 chlamydospores/tuber), and plant growth promoting fungus, *G. mosseae* (2 x 10^3 spores/tuber), followed by soil application of neem cake (500 kg/ha) in a farmer's field (naturally infested with *M. incognita* race 2 population) against root-knot nematodes. Plots that received a combination of neem cake amendment + treated bulbs (*P. chlamydosporia* and *G. mosseae*) recorded maximum per cent of healthy roots, flower spikes/m^2, spike length and number of florets/spike, and lowest number of root galls and nematode populations in soil and the root.

Rao and Gowen (1999) and Rao *et al*. (2000) demonstrated successful compatibility between mycorrhiza (*G. deserticola/G. mosseae*) and nematode antagonistic bacterium, (*Pasteuria penetrans)* in tomato and their combined suppression of *M. incognita* in glass house and field conditions.

Nagesh and Reddy (2004) in an attempt to elicit biochemical substantiation for the observed difference in resistance to nematode infection in roots colonized by mycorrhiza, and susceptibility of the fresh flush of roots of the same plant that escaped mycorrhizal colonization, observed that phenylalanine ammonia lyase (PAL) and indole acetic acid (IAA) oxidase activities were maximum in roots colonized by *G. fasciculatum* followed by the roots that received mycorrhiza plus juveniles of root-knot nematode, while the activity of polyphenol oxidase (PPO) was recorded to be minimum in these roots. The roots that received juveniles of root-knot nematode recorded minimum activity of PAL and IAA oxidase enzymes and maximum activity of PPO. Since, the roots of same plant that received mycorrhiza and that did not receive mycorrhiza; and the plant that received nematode alone and mycorrhiza plus nematode recorded differential biochemical contents of proteins, total phenols and IAA, and differential activities of enzymes under study, it was evident that the biochemical defense response to mycorrhizal colonization against root-knot nematodes was localized and not systemic. This explained for the response of plant that differed in root galling due to nematode infection in presence of mycorrhizal colonization. The new or fresh roots which missed mycorrhizal colonization, were infected by nematodes and developed root galls. With these observations, the authors optimized the dose of AM fungal application (biopriming the seedlings) in tomato nursery to a minimum of 8 chlamydospores per seedling in order to obtain at least 80% of the root with mycorrhizal colonization before transplanting in main field.

In the present study, biopriming the tobacco seedlings in nursery with *P. lilacinus* strain NBAII PL55*, G. fasciculatum* and their combinations, with soil application of neem cake or vermicompost had no adverse effects on seed germination, and in turn exhibited a beneficial effect in terms of obtaining higher number of healthy transplants of tobacco, relatively free of root-knot nematode infection and significantly reduced nematode infection in roots and populations in treated soil.

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