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Evaluation of *Paecilomyces lilacinus* for the Management of Root-Knot Nematode, *Meloidogyne incognita* in Flue Cured Virginia (FCV) Tobacco Nursery

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ABSTRACT: Flue Cured Virginia (FCV) tobacco is a major commercial crop grown in light soil regions of Karnataka. Root-knot nematodes, *Meloidogyne incognita* is a major threat to the successful production causing heavy yield and quality loss. Nematicide like carbofuran is being used against this nematode, but with limited efficacy. Extensive use of pesticides of chemical origin especially in higher doses for nematode control has to be avoided due to higher costs and associated hazards to the environment. Replicated trials were conducted with varied dosage levels of *Paecilomyces lilacinus* against the nematode in FCV tobacco nursery raised in root-knot nematode sick plots. Results revealed that at the time of final pulling, *P. lilacinus* @ 30g/m^2 recorded significantly increased total healthy transplants count of 857.5/m^2 and was on par with *P. lilacinus* @ 25g/m^2 (845.1/m^1. Similarly at 60 DAS, applications of *P. lilacinus* @ 30g/m^2 and @ 25g/m^2 were on par with each other in recording the significantly reduced RKI of 1.81 and 1.88 respectively. Both the treatments were also on par with combined application *P. lilacinus* @ 30g/m^2 + Carbofuran @ 10g/m^2, which recorded the significantly reduced RKI of 1.81 compared to untreated check (3.86).

Key words: *Paecilomyces lilacinus,* carbofuran, *Meloidogyne incognita,* FCV tobacco nursery.

Flue-Cured Virginia (FCV) tobacco is an important commercial crop grown in Karnataka light soils (KLS) as rainfed crop has lot of export potentiality. KLS tobacco is preferred internationally due to its ideal chemistry with below detectable levels of TSNA (Tobacco specific nitrosamines) compounds and pesticides residues. Among the various pest and diseases associated with the crop, plant parasitic nematodes and particularly the root-knot nematode, *Meloidogyne incognita* pose serious threat for the production and productivity causing significant reduction in terms of both yield and quality of the tobacco crop in nursery and main field to the tune of 59.4% and 52.9% respectively (Hussaini, 1983; Ramakrishnan *et al.,* 2001). Root-knot nematode infested seedlings, when transplanted in main field, exhibit stunted growth and may even collapse tesulting in gaps. Losses caused by this nematode are very high, especially when they interact with other disease causing pathogens. Ramakrishnan *et al.* (2008) had reported that root-knot nematode, *M. incognita* predispose FCV tobacco crop to wilt disease caused by *Fusarium oxysporum f.* sp. *nicotianae* contributing to significantly reduced yields. Both fumigant and non-fumigant nematicides such as dazomet and carbofuran have been successfully used against this nematode in nursery (Ramakrishnan *et al.,* 1998). But excessive use of synthetic pesticides is not cost effective and moreover many effective nematicides were withdrawn from the market due to their ill-effects and hazards they pose to environment. FCV tobacco. grown in Karnataka being an exportable commodity, presence of chemical residues is a great concern and hence use of such nematicides is highly discouraged. Bio-management of root-knot nematode with antagonistic organisms is an alternative, cost effective and eco-friendly approach. Biological control of plant parasitic nematodes is important in view of long-term advantage of management. *Paecilomyces lilacinus* is the potential egg parasitic fungus, which is highly safe and has been used successfully to manage root-knot nematodes in various crop plants (Ekanayake & Jayasundara, 1994; Jonathan *et al.,* 1995;

Ramakrishnan & Nagesh, 2011). The fungus affects the egg masses, engulfs and penetrates the eggs and proliferates within them by consuming the egg contents (Zaki & Bhatti, 1990). Hence, an attempt was made to evaluate commercial formulation of *Paecilomyces lilacinus* (2 x 10^6 spores/g) in varied dosage levels and also in combination with carbofuran under replicated trials for the management of root-knot nematode, *M. incognita* in FCV tobacco nursery.

MATERIALS AND METHODS

The replicated nursery trials for two seasons were conducted during 2011 & 2012 in the root-knot nematode infested nursery site of CTRI Research Stattion, Hunsur. Raised nursery beds each of 1.2 m X 1.2m size were prepared to fine tilt and the mean initial population of infective juveniles of *M. incognita* was 171 J2/100g soil. The commercial formulation of the egg parasitic fungi, Jai V Jai -*P. lilacinus* formulated as wettable powder with a spore load 2 x 10^6 spores/g formulations was incorporated into the raised nursery beds as per treatment schedule. The treatments incorporated includes, Jai V Jai -*P. lilacinus* at varied dosage levels (ie) @ 5, 10, 15, 20, 25 & 30g/m^2, Neem cake @ 400g / m^2 + Soil Solarization, carbofuran @ 10 g/m^2, Jai V Jai -*P. lilacinus* @ 30g / m^2 + carbofuran @10g / m^2 and with one untreated check. The treatments were replicated thrice in Randomised Block Design. For the treatment Neem cake @ 400g / m^2 + Soil Solarization, where soil solarisation was involved, after the incorporation of neem cake, prepared nursery beds were irrigated and covered with clear low density polyethylene (LDPE) film of 25 µm thickness when the moisture levels in the beds were around field capacity. Edges of the sheets were sealed with mud and left undisturbed for six weeks period. Then the sheets were removed and after two to three days of waiting period, FCV tobacco seed of variety "Kanchan", susceptible to root-knot nematode was sown at the rate 0.3g/ m^2 in all the nursery beds. All the other agronomic practices were followed as recommended. Observations such as Germination count (at 10 DAS), Count of healthy transplants at 60 DAS and at final pulling, root knot index (RKI) at 60 DAS and at final pulling were recorded. Germination count was taken at 15 DAS at random in ten squares, each with dimension of 100 cm^2, from which mean was calculated. Twenty five seedlings were examined for root-knot infection per replication in each treatment and graded under 0-5 scale, from which mean RKI was calculated. In addition, number of galls/g. root, egg mass/g. root and eggs/egg mass were also recorded. Soil samples were drawn before the application of treatments (initial) and at the end of the experiment (final) for the estimation of infective nematode population in the soil. Data gathered were statistically analysed using standard procedure.

RESULTS AND DISCUSSION

All the treatments evaluated under nursery conditions were found to be significantly superior over check in both reducing the root-knot disease incidence and in subsequently improving the seedling growth and root knot free seedlings count. Data from Table 1 revealed that, there was no adverse effect of the product, Jai V Jai -*P. lilacinus* on FCV tobacco seed germination. At 45 DAS, *P. lilacinus* @30g/m^2. significantly differed from the other dosage levels and increased the mean healthy transplantable seedlings count to 316.5/m^2 compared to 216.0/m^2 in untreated check. Whereas, at 60 DAS, *P. lilacinus* @ 30g/m^2 recorded maximum number of mean healthy transplantable seedlings count (320.3/m^2) compared to check (237.1/m^2). But, at the time of final pulling, *P. lilacinus* @ 30g/ m^2 recorded significantly increased mean total healthy transplants count of 857.5/ m^2, which is 53.7 per cent increase over check. It was also on par with *P. lilacinus* @ 25g/m^2, which recorded mean total healthy transplantable seedlings count of 845.1/m^2, which is 51.5 per cent increase over untreated check (557.8/m^2). Similar yield increase in okra plants grown in root-knot sick soil due to soil application of *P. lilacinus* was reported by Dhawan *et al*. (2004). Similar to the present results, Nagesh *et al.* (2001) had obtained significantly increased yield of chrysanthemum flowers to the tune of 23 -28% by use of talc-formulation of *P. lilacinus* @ 4 -6 kg/ac against root-knot nematodes. In the present investigations, carbofuran @ 10g/m^2, the recommended nematicide increased the total healthy transplantable seedlings count to the tune of 22.8 per cent only compared to check. Whereas its combined application with *P. lilacinus* @ 30g/m^2 recorded the maximum total healthy transplants count to the tune of

Table 1. Effect of *Paecilomyces lilacinus* on root-knot free and healthy transplants counts in FCV tobacco nursery.

Treatment details	Germ. count	Healthy transplants count (45 DAS)	% inc. over check	Healthy transplants count (60 DAS)	% inc. over check	Total healthy transplants count	% inc. over check
Paecilomyces lilacinus (2 x 10^6 cfu/g) @5g/m^2	21.1	235.6	9.0	292.8	23.5	658.0	18.0
P. lilacinus (2 x 10^6 cfu/g) @10g/m^2	21.8	240.0	11.1	281.5	18.7	655.5	17.5
P. lilacinus (2 x 10^6 cfu/g) @15g/m^2	21.6	235.1	8.8	294.0	23.9	682.3	22.3
P. lilacinus (2 x 10^6 cfu/g) @20g/m^2	20.3	273.6	26.6	313.1	32.1	759.1	36.1
P. lilacinus (2 x 10^6 cfu/g) @25g/m^2	21.2	307.5	42.3	336.1	41.7	845.1	51.5
P. lilacinus (2 x 10^6 cfu/g) @30g/m^2	21.1	316.5	46.5	320.3	35.1	857.5	53.7
Neem cake @400g/m^2 + Soil Solarization	21.7	278.0	28.7	303.0	27.7	676.0	21.1
Carbofuran @10g/m^2	21.2	279.0	29.1	295.5	24.6	685.0	22.8
P. lilacinus (2 x 10^6 cfu/g) @30g/m^2 + Carbofuran @ 10g/m^2	20.5	313.1	44.9	336.8	42.0	860.8	54.3
Control	20.3	216.0	-	237.1	-	557.8	-
S.Em	0.78	1.84		3.92		3.16	
CD (P=0.05)	NS	5.10		10.86		8.77	

54.3 per cent and it was on par with both the best dosage levels (25 &30 g/m^2) of *P. lilacinus.*

At the time of final pulling, data from table 2 revealed that application of *P. lilacinus* @ 30 g/m^2 and 25 g/m^2 were on par with each other in significantly reducing the number of egg mass/g. root to the tune of 31.0 and 26.5 per cent respectively and eggs/egg mass to the tune of 27.0 and 24.6 per cent respectively and final soil population/100 g soil to the tune of 52.5 and 49.6 per cent respectively compared to un treated check. In general, the application of *P. lilacinus* at varied dosage levels from 5 to 30g/m^2 in FCV tobacco nursery against root-knot nematodes caused 16.5 to 31.0 per cent reduction in number of egg mass/g root, 13.7 to 27.0 per cent reduction in number of root-knot nematode eggs/egg mass and 30.2 to 52.5 per cent reduction in final soil nematode population as compared to un treated check. Similar decrease in root-knot nematode soil population due to application of *P. lilacinus* in banana was also reported earlier by Jonathan & Rajendran (2000). At 45 DAS, application *P. lilacinus* @ 30g/m^2 in FCV tobacco nursery beds significantly reduced root-knot index (RKI) to 1.30 on 0-5 Scale, which is 50.1% decrease over untreated check (2.61) and was found on par with *P. lilacinus* @ 25g/m^2 (1.31). Similarly at 60 DAS, applications of *P. lilacinus* @ 30g/m^2 and @ 25g/m^2 were on par with each other in recording the significantly reduced RKI of 1.81 and 1.88 respectively. Decrease over untreated check was 53.1 and 51.3 per cent respectively. Both the best treatments were also on par with combined application *P. lilacinus* @ 30g/m^2 + Carbofuran @ 10g/m^2, which recorded the significantly reduced RKI of 1.81 compared to untreated check (3.86). Similarly Reddy & Khan (1988) reported 76% reduction in population of reniform nematode, *Rotylenchulus reniformis* with combined application of carbofuran at 2 kg a.i/ha and *P. lilacinus* @ 2g on tomato. Saikia & Das (2001) got highest reduction in galls (60%) with combined application of *P. lilacinus* @ 2g/kg soil and carbofuran @ 1kg. This clearly exhibits the compatibility of *P. lilacinus* with the chemical

Table 2. Effect of *Paecilomyces lilacinus* on root knot nematode multiplication in FCV tobacco nursery .

Treatment details	RKI at 45 DAS	% Dec. over check	RKI at 60 DAS	% Dec. over check /g. root	No.of Egg masses	% Dec. over check	No.of Eggs/egg mass	% Dec. over check	Soil population (/100 g. Soil)	% Dec. over check
Paecilomyces lilacinus (2 x 10^6 cfu/g) @5g/m^2	2.30	11.8	3.03	14.5	16.3	18.5	250.0	14.8	99.5	31.8
P. lilacinus (2 x 10^6 cfu/g) @10g/m^2	2.38	8.8	3.13	18.9	16.7	16.5	253.0	13.7	101.8	30.2
P. lilacinus (2 x 10^6 cfu/g) @15g/m^2	2.33	10.7	2.95	23.5	16.0	20.0	245.5	16.3	101.0	30.8
P. lilacinus (2 x 10^6 cfu/g) @20g/m^2	2.30	11.8	2.60	32.6	15.4	23.0	242.5	17.4	87.3	40.2
P. lilacinus (2 x 10^6 cfu/g) @25g/m^2	1.31	49.8	1.88	51.3	14.7	26.5	235.0	19.9	73.5	49.6
P. lilacinus (2 x 10^6 cfu/g) @30g/m^2	1.30	50.1	1.81	53.1	13.8	31.0	214.1	27.0	69.3	52.5
Neem cake @400g/m^2 + Soil Solarization	2.26	13.4	2.45	36.5	16.5	17.5	242.5	17.3	93.8	35.7
Carbofuran @10g/m^2	2.00	23.4	2.25	41.7	15.7	21.5	242.0	17.5	82.6	43.4
P. lilacinus (2 x 10^6 cfu/g) @30g/m^2 + Carbofuran @ 10g/m^2	1.27	51.3	1.81	53.1	13.5	32.5	214.5	26.9	69.0	52.7
Control	2.61	-	3.86	-	20.0	-	293.5	-	146.0	-
S.Em	0.06		0.03		0.54		1.62		1.80	
CD (P=0.05)	0.16		0.08		1.49		4.49		4.98	

nematicide, carbofuran. Moreover, both the best treatments in the present study were also significantly superior to sole application of carbofuran @ 10g/m^2 with RKI of 2.25 in reducing the root-knot disease incidence in FCV tobacco nursery. The experimental results clearly indicate that *P. lilacinus* @ 30g/m^2 was on par with *P. lilacinus* @ 25g/m^2 in reducing the root knot nematodes incidence in FCV tobacco nursery and in subsequently increasing the total root-knot free and healthy seedlings count. Hence, it is concluded that, application of *P. lilacinus* in talc-formulation with spore load of (2 x 10^6 cfu/g) @ 25g/m^2 (ie) 3 kg/unit nursery (120m^2) is an ideal dosage for the effective management of root-knot nematodes in FCV tobacco nursery.

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