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COLEUS FORSKOHLII: BIOMANAGEMENT OF ROOT KNOT NEMATODE BY BIOCONTROL FUNGI

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ABSTRACT

Meloidogyne incognita (Root-knot nematode) is an important pest on *Coleus forskohlii* in India. Nematode diseases are serious because they attack the roots of *Coleus*, which are the source of drug of commerce. The tubers possess medicinal properties and significantly rich source of both enzymatic and non-enzymatic antioxidants. The active ingredient (forskolin) a labdane diterpene, is an adenylyl cyclase activator and cAMP stimulator, used in research studies by medical professionals and pharmacologists. Investigations were carried using different strains of biocontrol fungi along with some market formulations to evaluate their plant growth promoting and antinematicidal properties. Plant growth characters like shoot and root length, shoot and root weight, number of tubers, tuber length and Root Knot Index per plant were studied. Among the different strains of biocontrol fungi and the locally isolated strains of biocontrol fungi *T. harzianum 2 (*Jaipur*)* and *Paecilomyces* gave best results and were equally effective to their screened commercial market formulations. There was an increase in plant growth characters, tuber yield and reduction in root knot disease, over untreated plants, presenting a safe, economic and efficient method for managing root knot disease on *Coleus forskohlii.*

KEYWORDS: *Coleus forskohlii, Meloidogyne incognita*, Root-knot nematode, Biocontrol fungi, Plant growth characters.

INTRODUCTION

Microorganisms that can grow in the rhizosphere provide the front line defense for roots against pathogen attach and are ideal for use as biocontrol agents. Fungi and bacteria are two major groups of microbes which are abundant in soil and some of them have showed great

potential as bio control agent's nematodes.[1] Fungi constitute a major group of bioagents for control of various plant pathogens including fungi. Biocontrol of pathogens through antagonists has assumed importance in recent years. The management of parasitic nematodes by bioagents is the most successful method of nematode control in plants.[2]

The effect of application of different biocontrol agents (*Trichoderma* spp (eight isolates), *Pseudomonas fluorescens* (four isolates) and *Bacillus subtilis* (two isolates)) was tested against root-knot nematode, *Meloidogyne incognita* complex diseases.[3] The efficacy of biocontrol agents (*Paecilomyces lilacinus* and *Cladosporium oxysporum*) and/or oil cakes of castor, linseed, groundnut, mahhua and neem was evaluated in the management of root knot nematode, *Meloidogyne javanica* infecting eggplant under glasshouse conditions. All the treatments effectively suppressed the nematode population and kept the infection at significantly low level.[4] The biocontrol activity of *Trichoderma* and *Gliocladium virens* was studied on *Fusarium oxysporum* f.sp. *cumini* causing wilt of cumin.[5] The compatibility of biocontrol fungi and neem products have been evaluated for the management of nematode disease infecting *Cajanus cajan*.[6,7]

Nematode fungal disease complex management involving *Meloidogyne incognita* and *Macrophomina phaseolina* in *Coleus forskohlii*. *T. viride* and on tuber yield in medicinal Coleus has been studied.[8] Treatments involving neem leaves, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, or aldicarb have reduced the suppressive effect of the nematode and has found significant increase in the dry matter production and yield of inoculated plants compared to the inoculated control.[9] Application of *Paecilomyces lilacinus* on management of root knot infection in *Vigna radiate*.[10] The number of galls, egg masses and final nematode population of *M. incognita* was studied in okra plants treated with *T. harzainum* as a seed treatment and/or soil application.[11]

Culture filtrate of *Aspergillus niger* F22 were evaluted against *Meloidogyne incognita* to observe mortality of second-stage juveniles (J2s) and inhibition of egg hatching.[12]

The biocontrol activity of *T. harzianum* against *M. incognita* was determined to observe the (i) mycoparasitism of *T. harzianum* on *M. incognita* eggs and (ii) bio-efficacy of *T. harzianum* against *M. incognita* on okra under pot condition. *Trichoderma harzianum* parasitism on *Meloidogyne incognita* eggs and juveniles under Assam condition was also studied. *M. incognita* egg masses, *T. harzianum* was applied either as a seed treatment and/or

soil application or both. Carbosulfan was used as a seed treatment and carbofuran as soil application was applied as chemical checks both either singly or in combination.[13]

MATERIALS AND METHODS

Earthen pots were surface sterilized and filled with autoclaved soil. Terminal cuttings of coleus (which were surface sterilized) were planted at the rate of one plant per pot. Five replications were maintained. After establishment of the cuttings the biocontrol agents in their sequence were applied in soil.

Fungal culture and inoculation: The biocontrol fungi, *Trichoderma* spp., *Gliocladium* and *Paecilomyces* was grown on PDA medium for 15 days at 28±2°C in BOD incubator. Pure culture of fungus prepared on PDA, was further multiplied on PD Broth, the mycelia mat was filtered, macerated in distilled and sterilized water. This was again filtered though Whatman filter paper to get spores only. Number of spores per ml was counted with the help of haemocytometer and cfu was maintained at 8X 10^6spores/ml.

T-1=*Trichoderma harzianum* 1 (Jaipur)

T-2=*Trichoderma harzianum* 2 (Jaipur)

T-3=*Trichoderma harzianum* 3(Jaipur)

T-4=*Trichoderma harzianum* 4(Jaipur)

T-5=T*richoderma harzianum* 5(Jaipur)

T-6=*Trichoderma harzianum* (Udaipur)

T-7=*Trichoderma aureoviride*

T-8=*Trichoderma hamatum*

T-9= *Gliocladium virens*

T-10=*Paecilomyces*

Nematode culture and inoculation: Root-knot nematode, *M. incognita* was isolated from the coleus infected plants. Pure culture was multiplied on brinjal plants. Just before the inoculation, the feeder roots of the seedlings (10 days old), were exposed by carefully removing, the adhering top layer of the soil. The required quantity of nematode suspension having 1000 freshly hatched juveniles was poured uniformly all over the exposed roots of coleus and covered immediately with the top soil. This was followed by light watering of the plants.

The experiments were terminated 160 days after inoculation. Observations were recorded on shoot length, root length, shoot weight, root weight, tuber length, no. of tuber, no. of galls. On its basis the root knot index and resistance rating was given.

To observe the influence of inoculum sequence of biocontrol fungi on root knot infection on coleus the following experiment was conducted

- Biocontrol fungi(15 days before) +Nematode (N)
- Biocontrol fungi (simultaneously) + Nematode (N)
- Biocontrol fungi (15 days later)+Nematode (N)
- Nematode alone
- Control

RESULTS

Table: Effect of different inoculum sequence of biocontrol fungi in the biomanagement of root knot disease (*Meloidogyne incognita*) in *Coleus forskohlii* Briq.

S.No.	Treatments	Length (cm)		Weight (gm)			Tuber Length(cm)	No. of tubers	No. of galls	R.K.I
		Shoot	Root	Shoot	Fresh	Root Dry				
1.	T1(15 DB)	39.00	35.67	110.33	14.00	7.00	20.33	13.33	20.33	2.33
	T1 (S)	37.00	30.33	103.67	13.33	6.33	19.33	12.67	25.67	2.67
	T1(15 DL)	35.33	28.00	93.00	12.67	5.67	17.65	10.35	48.67	3.67
2.	T2(15 DB)	39.33	36.00	136.67	14.67	7.33	22.00	14.33	5.00	2.00
	T2(S)	37.67	32.33	120.00	13.00	6.65	18.67	13.67	18.67	2.33
	T2(15 DL)	34.33	30.67	117.00	12.67	5.67	16.00	12.33	30.00	2.67
3	T3(15 DB)	38.67	30.67	120.00	13.67	6.33	20.67	12.67	18.00	2.67
	T3(S)	36.33	27.33	110.33	12.33	6.00	18.33	10.33	29.00	2.67
	T3(15 DL)	30.00	25.00	95.00	11.67	5.67	17.67	11.67	48.33	3.67
4	T4(15 DB)	37.00	32.67	99.67	13.33	6.33	18.33	13.00	22.00	2.33
	T4(S)	36.67	28.67	93.67	12.33	6.00	17.67	11.33	35.00	3.33
	T4(15 DL)	33.33	25.33	85.00	11.33	5.67	14.33	8.67	53.67	3.67
5	T5(15 DB)	37.67	30.33	100.67	13.67	6.33	19.67	13.00	28.33	2.67
	T5(S)	36.00	28.67	98.33	12.00	6.00	16.33	11.67	45.00	3.67
	T5(15 DL)	32.67	25.33	84.00	11.67	5.67	14.00	10.00	65.00	3.67
6	T6(15 DB)	38.67	34.67	123.67	14.00	7.00	20.33	13.67	18.33	2.33
	T6(S)	37.33	30.00	118.00	13.67	6.00	18.00	12.00	29.67	2.67
	T6(15 DL)	35.00	27.67	110.33	12.33	5.67	16.33	10.67	60.67	3.67
7	T7(15 DB)	37.00	30.33	85.00	13.00	6.00	20.00	12.00	28.00	2.67
	T7(S)	35.67	28.67	78.67	12.67	6.00	17.67	11.33	42.33	3.33
	T7(15 DL)	32.33	27.33	75.33	11.67	5.67	14.33	9.67	72.00	3.67
8	T8(15 DB)	36.33	32.67	87.33	13.67	6.33	18.67	13.33	30.33	3.00
	T8(S)	32.67	29.33	82.67	12.67	6.00	16.00	11.33	48.67	3.33
	T8(15 DL)	30.33	27.00	78.33	11.33	5.67	14.67	10.00	70.00	3.67

9	T9(15 DB)	37.00	33.00	95.67	13.33	6.33	19.67	13.67	20.00	2.00
	T9(S)	34.67	29.00	89.33	12.33	5.67	17.00	12.33	30.00	3.00
	T9(15 DL)	32.67	27.67	82.00	11.33	5.33	15.67	11.67	60.00	3.33
10	T10(15 DB)	38.67	35.00	130.00	14.33	7.33	21.67	14.67	11.33	2.00
	T10(S)	37.33	32.00	110.67	13.67	6.67	19.33	12.67	16.75	2.67
	T10(15 DL)	34.00	29.33	100.33	12.00	6.33	16.67	11.33	25.00	2.67
11	Nematode alone	28.67	22.33	48.67	11.00	5.33	7.33	7.67	110.00	5.00
12	Control	40.00	36.00	140.00	15.00	7.46	22.00	20.00	0.00	0.00
	CD at 5%	1.530	2.753	5.310	1.024	0.651	1.295	0.994	3.000	0.135

T-1=*Trichoderma harzianum* 1(Jaipur) T-2=*Trichoderma harzianum* 2 (Jaipur)

T-3=*Trichoderma harzianum* 3(Jaipur) T-4=*Trichoderma harzianum* 4(Jaipur)

T-5=*Trichoderma harzianum* 5(Jaipur) T-6=*Trichoderma harzianum* (Udaipur)

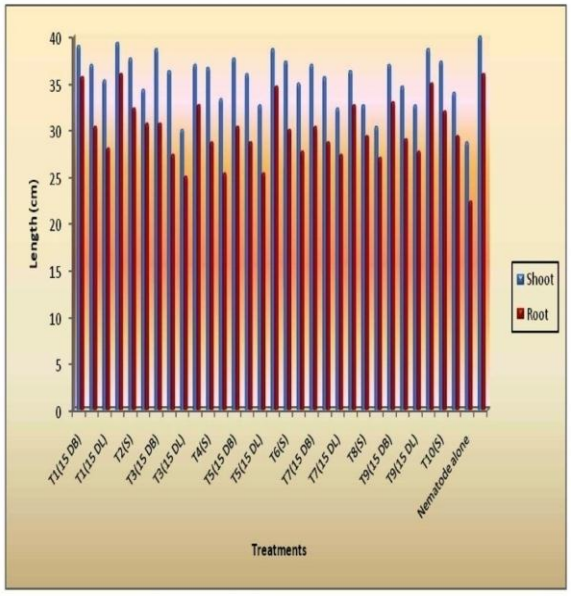
T-7=*Trichoderma aureoviride* *T-8=Trichoderma hamatum*

T-9= Gliocladium virens *T-10=Paecilomyces*

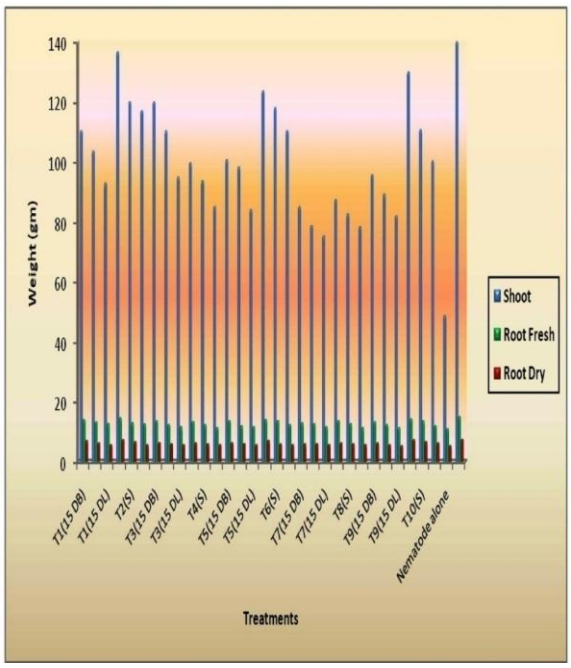
DB= BIOCONTROL FUNGTI-15 DAYS BEFORE NEMATODE S=BIOCONTROL FUNGI-NEMATODE SIMULTANEOUSLY DL= BIOCONTROL FUNGI-15 DAYS LATER NEMATODE

Effect of different inoculum sequence of biocontrol fungi in the biomanagement of root knot disease (*Meloidogyne incognita*) in *Coleus forskohlii* Briq.

Graph-20

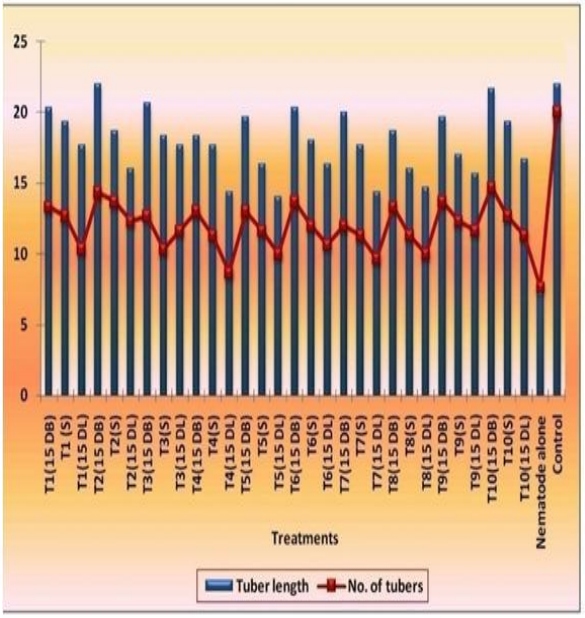


Graph-21

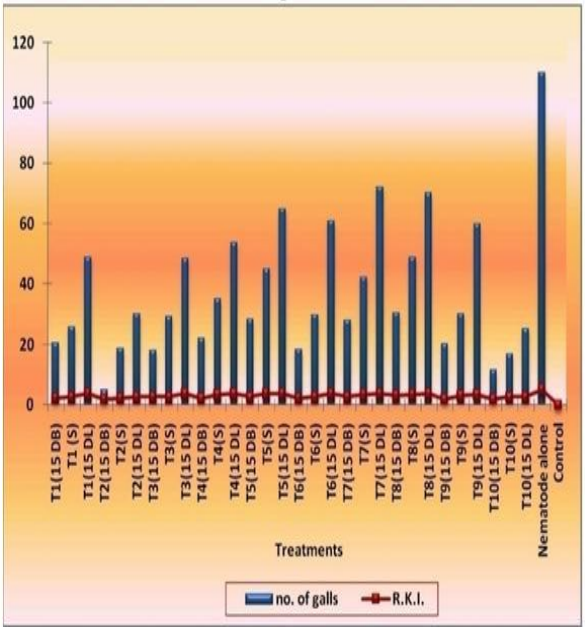


Effect of different inoculum sequence of biocontrol fungi in the biomanagement of root knot disease (*Meloidogyne incognita*) in *Coleus forskohlii* Briq.

Graph-22



Graph-23



All the treatments generally showed improvement of plant vigor and reduced the number of galls as compared to nematode alone treated plants.(Table and Graphs) Best results were obtained by *Trichoderma harzianum* 2 (Jaipur) and *Paecilomyces lilacinus* treatments in increasing plant growth characteristics (shoot and root length and weight, tuber length and their numbers) and reduced number of galls. *Trichoderma harzianum* 1 (Jaipur) and

Trichoderma harzianum (Udaipur) treatments also gave good results. Treatments of biocontrol fungi when given 15 days before nematode inoculation gave the best results, as compared simultaneous treatment or 15 days later treatment.

DISCUSSION

The control of soil-borne plant parasitic nematodes using trapping fungi is of great interest because the use of nematicides and soil sterilants brings about various ecological and toxicological dangers.[14] *Trichoderma* spp. is among the most promising biocontrol agents.[15]

Bio-management of Root-Knot Nematode, *Meloidogyne incognita* on *Coleus forskohlii* Briq using *Trichoderma viride* and recorded increased plant growth and reduced nematode population compared to control.[16,17,18,19] Biocontrol fungi *Arthrobotrys oligospora, P. lilacinus, V. chlamydosporium, Gliocladium virens, T. viride* reduced the multiplication of *M. incognita* and resulted in improved growth of tomato plant.[20] The lowest number of galls, egg masses and final nematode population of *M. incognita* was recorded in okra plants treated with *T. harzainum* as a seed treatment and/or soil application.[11] Nematophagous fungus *P. lilacinus* also has biocontrol potential against root -knot infection It was also observed by several workers in other plants that *Paecilomyces* was highly efficient as biocontrol agent.[3,21] Our results are supported by researchers on *Cajanus cajan* L. who have successfully managed rootknot infection by using different biocontrol fungi 15 days prior to nematode.[6,7]

Paecilomyces lilacinus / Verticillium chlamydosporium in the presence of neem cake and neem seed powder and rational integration of these components would help in the increased control of *M. incognita, Rotyenchulus reniformis*.[22] Nematophagous fungus *P. lilacinus* also has biocontrol potential against root knot. It was also observed by several workers that *Paecilomyces* was highly efficient as biocontrol agent.[9] The disease control efficacy of the mixture of F22-WP10 + oxalic acid-WP8 was significantly higher than that of a chemical nematicide, Sunchungtan (ai 30% fosthiazate). These results suggest that *A. niger* F22 can be used as a microbial nematicide for the control of root-knot nematode disease.[12]

It has been observed that hypal parasitism and production of inhibitory substances by different species of *Trichoderma* has been responsible for inhibitory effect against the pathogen. Use of these microorganisms exhibited varied growth responses from inhibitory,

stimulatory, and neutral to different pesticides, amongst which *Trichoderma* spp. indeed had ability to control plant pathogens.[8,9,16,18,19]

Biocontrol potential of *Trichoderma* has been studied extensively. The wide range of application of *Trichoderma* sp. is due to the various antagonistic mechanisms found in different *Trichoderma* isolates enabling them to function as potent biocontrol agents on many different crops, against a wide range of pathogens and in several ecological situations. Many workers have supported the effective results of *Trichoderma* and observed that incorporation of *Trichoderma* sp. significantly improved plant growth in comparison to control and nematode alone inoculated plants.[8,9,13,16,18,19]

The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which based on the activation of multiple mechanism.[23]

In recent research, the conidia of the *T. harzianum* were found inside of the eggs and attached to the J2s with the gelatinous matrix. The eggs were penetrated and parasitized by the hyphae of *T. harzianum*, while eggs containing juveniles were also parasitised by *T. harzianum* results showed that either *T. harzianum* or the chemicals (Carbofuran and Carbosulfan) when applied together as a seed treatment and soil application, improved plant growth parameters of okra and reduced the nematode multiplication as compared to when they were applied either as a seed treatment or soil application. Application of chemicals either as a seed treatment or soil application emerged as the most effective treatment as compared to the *T. harzianum*. However, in respect of *T. harzianum* when applied together as a seed treatment and soil application showed significantly better results in an improving the plant growth parameters and reduction in the nematode multiplication as compared to the treatments with carbosulfan as a seed treatment and carbofuran as soil application alone the use of biocontrol agent' *i.e. T. harzainum* may be an effective alternative to the chemicals when applied together as a seed treatment and soil application showed significantly better results in an improving the plant growth parameters and reduction in the nematode multiplication as compared to the treatments with carbosulfan as a seed treatment and carbofuran as soil application alone for the management of *M. incognita* infected on okra under Assam condition.[13] The previous researches support and confirm our results.

Therefore, overall increase in plant characters observed by biocontrol fungi treatments over nematode alone treatment. Therefore, we recommend 15 days prior application of *Trichoderma harzianum* 2 (Jaipur) and *Paecilomyces lilacinus* treatments for improved plant growth, tuber yield and reduced root knot disease.

CONCLUSION

Among the different strains of biocontrol fungi, bacteria and vam, the locally isolated strains of biocontrol fungi *T. harzianum 2 (*Jaipur*)*and *Paecilomyces* proved to be the best. They were equally effective to their screened commercial market formulations. There was an increase in plant growth characters, tuber yield and reduction in root knot disease, over untreated plants, presenting a safe, economic and efficient method for managing root knot disease on *Coleus forskohlii.* Bicontrol fungi are the best management practice for nematode management

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