



First Indigenous technical knowledge using antagonistic fungi, *Paecilomyces lilacinus* along with molasses for control root-knot nematode, *Meloidogyne incognita* infesting Cucurbitaceous crop.

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Abstract

The sick plot was splitted into two blocks i.e., treated and untreated each having 10 sub plots each of size 3mx3m serving as replication. In the treated plots, antagonistic fungi, *Paecilomyces lilacinus* @ 2 kg a.i/ha application was done, before sowing of Cucumber seeds was followed by light irrigation. The crop was grown following agronomic package and practices. The experiment was terminated, 100 after sowing and observations were recorded on plant growth parameters as well as on the nematode population development in both inoculated and uninoculated plots.

Keywords: *Meloidogyne incognita*, Cucumber, Banki Local, *Cucumis sativus* L., yield loss, plant growth parameters

Introduction

Root-knot, *Meloidogyne incognita* and like many other obligate parasites are capable of disturbing the host metabolism. The changes in the physiological and biochemical processes of infected host as a consequence of disturbed metabolism decide whether the host becomes susceptible or resistant to nematode attack (Krusberg, 1963). In this context an intimate knowledge of nematode physiology and biochemistry along with its host is absolutely essential for developing plant resistance against the nematodes. In the recent past some progress has also been made in this direction to understand the basic biochemical mechanism of plant-nematode interactions by several workers (Ganguly and Dasgupta, 1983; Howell and Krusberg, 1966; Mohanty, *et al.*, 1995; Nayak, 2015).

The fungus *Paecilomyces lilacinus* (Thorn) Samson has been reported as a potential biological control agent for root-knot nematodes and other plant-parasitic nematodes. Antagonistic fungi, *Paecilomyces lilacinus* is a common soil hyphomycete. It parasitizes eggs of *Meloidogyne* spp. and *Globodera* spp. This fungus also invades the females or cysts of a number of nematode species. It exhibits chitinase activity when grown on chitinagar plates and produces a peptidal antibiotic which has wide antimicrobial activity against fungi, yeast, and gram-positive bacteria.

Antagonistic fungi, *P. lilacinus* colonizes *M. incognita* eggs, preventing them from hatching and leaving fewer juveniles to penetrate root tissue. Also, the finding that *P. lilacinus* colonizes the root tissue as an epiphyte and endophyte contributes to our understanding of the mechanism of biological control against root-knot nematodes when roots of tomato or possibly other susceptible crop plants are treated with this fungus prior to planting.

Recent advances in molecular biology and genetic engineering have ushered in a new era equipping scientists with the power to tailor the biological system at will through recombinant DNA-technology. This technology would be of immense utility in introducing pesticidal gene into root colonizing organisms including nematodes. In this context full knowledge of nematode physiology, biochemistry along with its host is absolutely essential. Biochemical changes induced by plant parasitic nematodes relating to various crops have been well documented in a series of publications (Mohanty et al., 2001, Farooqi, et.al., 1988, Mote, et.al., 1990, Sundarraj and Meheta, 1991. A series of biochemical and physiological reactions occur in host plants in response to root-knot nematode infection as a result of which, the plant is either overcome by the nematode and the disease ensues or the nematode is localised by the plant and disease development is limited. Detailed characterization of these biochemical and physiological processes is essential to advance our understanding of plant-nematode interaction. This information would be of greatly helpful to plant breeders and nematologists in breeding works for development of cultivars resistant to root-knot nematode.

Materials and Methods

First Indigenous technical knowledge using antagonistic fungi, *Paecilomyces lilacinus* along with molasses for control root-knot nematode, *Meloidogyne incognita* infesting Cucumber, Banki Local.

An experiment was laid out in the sick plant, nearby KVK, Dhenkanal, naturally infested with root-knot nematode using pair plot technique to assess the avoidable yield loss of cucumber due to root-knot nematode, *Meloidogyne incognita*.

The field was thoroughly cultivated and pulverized soil samples (200c.c) were taken with the help of a hoe upto a depth of 15 cm and mixed thoroughly to prepare composite sample, one sample of 200 c.c was

taken and processed for nematode extraction by Cobb's sieving and decanting method (Cobb,1918) to know initial nematode population.

The sick plot was divided into two blocks i.e., treated and untreated each having 10 sub plots each of size 3mx3m serving as replication. For treated plots, *Paecilomyces lilacinus* @ 2 kg a.i/ha was applied before sowing of cucumber seeds followed by light watering. The crop was grown following agronomic package and practices. 100 days after sowing, the experiment was terminated and observations were recorded on plant growth parameters as well as on the nematode population development in both treated and untreated plots.

Recording of observations

Shoot Length

From above each plant height upto the top most portion, the shoot length was measured in scale.

Root Length

The root portion of each plant was cut, labeled and knots, if any, were opened followed by straightening of roots. The root length of each plant was measured upto tip in the meter scale.

Fresh Weight of Shoots And Roots

Fresh weight of shoot and root of individual plant were recorded in grams. These were further labeled and kept for recording of dry weights.

Dry Weight of Shoot and Root

Shoot and root of individual plants were air dried and kept in separate paper packets. Such packets were then placed in hot air oven at 70° C temperature for 48 hrs after which, the dry weights were recorded in gram.

Number of Galls

Total number of galls on roots of each plant was counted with the help of hand tally counter before drying and recorded as per root index in 1 to 5 point scale.

Estimation of *M. incognita* Population In Roots

At the time of harvest, roots of cucumber plants inoculated with *M. incognita* were lifted carefully. Infected root measuring one g from each replication of different treatments were tied separately with cotton threads and labeled accordingly. Then nematode in roots are stained by Byrd method.

Nematode Population in Soil

Soil from each plot was mixed thoroughly and 200ml sample from each plot was collected and screened by Cobb's sieving technique (Cobb, 1918) and modified Baermann funnel technique (Schindler, 1916) for estimation of nematode population in different treatments.

Yield

Yield from each plot were recorded at the time of termination of experiment. Data obtained were analysed according to 't' test for paired comparison at 5% level of probability.

Statistical Analysis

't' at error degree of freedom = $\frac{\bar{X}_1 - \bar{X}_2}{S_d}$

Where \bar{X}_1 = Mean yield of treated plot
 \bar{X}_2 = Mean yield of un treated plot

$$S_d = \frac{S}{\sqrt{n}}$$

Where S = Standard deviation =

$$\sqrt{\frac{\sum d^2 - \frac{(\sum d)^2}{n}}{n}}$$

n = number of paired plots.

d = differential value between two paired plots.

The avoidable yield loss and percent increase in yield over control (untreated) by following formulae. (Pradhan 1964).

Avoidable yield loss (%) =

$$\frac{\text{Mean yield of treated plot} - \text{Mean yield of untreated plot}}{\text{Mean yield of treated plot}} \times 100$$

Increase in yield (%) =

$$\frac{\text{Mean yield of treated plot} - \text{Mean yield of untreated plot}}{\text{Mean yield of untreated plot}} \times 100$$

Results and Discussion

The estimated direct crop losses due to phytonematodes ranged from 5 to 10 per cent of crop value annually. The information on monetary losses due to phytonematodes is essential for understanding the control measures. The experiment was conducted under field conditions to assess the avoidable yield losses due to root knot nematode on cucumber with paired plot design with soil application of antagonistic fungi, *Paecilomyces lilacinus* at 2 kg a.i./ha over untreated control. The results indicated that the avoidable loss in the yield of cucumber, Banki Local was recorded to be 74.52 per cent, when the crop was treated with *Paecilomyces lilacinus* at 2 kg a.i./ha.

The avoidable loss in the yield of cucumber ranged from 4.09 to 74.52 per cent. As the literature on assessment of yield losses due to root knot nematode in cucurbitaceous crops especially on cucumber is scanty the present findings are compared with some other vegetable crops. The loss of 46.92, 32.73 and 36.72 per cent in the yield of tomato, brinjal and bitter gourd, respectively due to root-knot nematode was also recorded by Darekar and Mhase (1988). The avoidable yield loss of 71.90 per cent in the yield of tomato due to *M. incognita* and 43.30, 41.80, 29.90 per cent in the yield of tomato brinjal and okra, respectively due to *M. javanica* was reported by Jain et al. (1994). Mote and Mhase (1997) also reported 27.20 per cent loss in yield of okra due to root-knot nematode, when the crop was treated with *Paecilomyces lilacinus* at 2 kg a.i./ha. Khanna and Kumar (2003) reported 22.9 to 42.8 per cent loss in yield of bitter gourd due to root knot nematode.

Conclusion

The assessment of avoidable yield losses due to root-knot nematode in cucumber indicated the loss in yield of cucumber to 74.52 per cent under field conditions, when the crop was treated with *Paecilomyces lilacinus* at 2 kg a.i./ha.

It can be inferred from the present study that the root knot nematode *Meloidogyne incognita* induce majorable biochemical changes in plants. Understanding the biochemical and molecular basis of plant nematode interaction will help us in identifying new targets to intervene with nematode parasitism and develop novel strategies to combat them. The information generated from this investigation can be manipulated through advanced biotechnological research for planning suitable management strategies.

Table 1. Effect of *Paecilomyces lilacinus* treatment on root and shoot length of cucumber, Banki Local infested with root-knot nematode, *M. incognita*

Replications	Root Length(cm)			Shoot Length(cm)		
	Treated (<i>Paecilomyces lilacinus</i> @ 2kg a.i/ha)	Untreated control	%increase Over control	Treated (<i>Paecilomyces lilacinus</i> @ 2kg a.i/ha)	Untreated Control	% increase over control
1	45.30	38.51	17.63	310.29	298.21	4.05
2	44.31	36.23	22.30	302.59	297.52	1.70
3	42.21	35.03	20.49	301.65	298.53	1.04
4	41.23	33.89	21.65	305.28	297.55	2.59
5	43.12	34.52	24.91	311.80	298.82	4.34
6	45.88	36.45	25.87	302.66	297.65	1.68
7	40.32	37.42	7.74	303.45	287.45	5.56
8	40.20	32.44	23.92	306.48	285.25	7.44
9	38.20	31.43	21.53	304.42	282.23	7.86
10	35.21	32.48	8.40	309.45	281.14	
SE(m)±	0.706			2.682		
t value	9.567			4.987		
CD(0.05)	8.03			15.903		

*Treated: (*Paecilomyces lilacinus*@ 2kg a.i/ha)**Table 2. Effect of *Paecilomyces lilacinus* treatment on fresh weight of root and shoot of cucumber, Banki Local infested with root-knot nematode, *M. incognita***

Replications	Fresh weight of root/plant (g)			Fresh weight of shoot/plant (g)		
	Treated (<i>Paecilomyces lilacinus</i> @ 2kg a.i/ha)	Untreated Control	% increase over control	Treated (<i>Paecilomyces lilacinus</i> @ 2kg a.i/ha)	Untreated control	%increase over control
1	50.70	42.75	18.59	125.21	98.2	27.5
2	51.24	41.55	23.32	125.35	118.21	6.04
3	50.10	42.45	18.02	123.75	121.35	1.97
4	48.75	36.45	33.74	124.81	120.85	3.27
5	45.23	38.23	18.31	128.72	125.75	2.36
6	42.21	31.31	34.81	141.51	122.45	15.56
7	40.55	30.45	33.16	123.58	119.21	3.66
8	40.23	31.25	28.73	119.83	99.35	20.61
9	45.23	32.45	39.38	112.86	98.25	14.87
10	41.11	32.35	27.07	102.92	94.48	8.93
SE(m)±	0.612			2.744		
t value	15.701			4.025		
CD(0.05)	11.425			13.132		

*Treated: (*Paecilomyces lilacinus*@ 2kg a.i/ha)

Table 3. Effect of *Paecilomyces lilacinus* treatment on dry weight of root and shoot of cucumber, Banki Local infested with root-knot nematode, *M. incognita*

Replications	Dry weight of root/plant (g)			Dry weight of shoot/plant (g)		
	Treated (<i>Paecilomyces lilacinus</i> @ 2kg a.i/ha)	Untreated control	%increase over control	Treated (<i>Paecilomyces lilacinus</i> @ 2kg a.i/ha)	Untreated control	% Increase Over control
1	19.51	11.95	63.26	81.75	72.75	12.42
2	20.75	12.93	60.47	82.35	72.45	13.66
3	20.01	13.75	45.52	83.78	73.85	13.44
4	17.85	10.23	74.48	81.25	72.45	12.14
5	20.07	12.30	63.17	80.35	71.23	12.80
6	17.02	10.75	58.32	84.25	74.25	13.46
7	18.25	11.00	65.90	85.31	75.35	13.21
8	17.75	10.88	63.14	83.48	73.42	13.70
9	17.25	11.09	55.54	84.45	74.41	13.49
10	16.85	10.85	55.29	82.23	72.41	13.56
SE(m)±	0.23			0.154		
t value	30.09			62.771		
CD(0.05)	9.78			11.493		

*Treated: (*Paecilomyces lilacinus*@ 2kg a.i/ha)**Table 4. Effect of *Paecilomyces lilacinus* treatment on yield of cucumber, Banki local infested with root-knot nematode, *M. incognita***

Replications	Yield in kg/m ² at termination		
	Treated (<i>Paecilomyces lilacinus</i> @ 2kg a.i/ha)	Untreated control	% increase over control
1	4.10	3.80	7.89
2	4.07	3.91	4.09
3	3.82	2.80	36.42
4	3.90	2.24	74.10
5	3.74	2.60	43.84
6	4.00	3.82	4.71
7	3.81	2.65	43.77
8	3.90	2.42	61.15
9	3.70	2.12	74.52
10	4.00	3.72	7.52
SE(m)±	0.192		
t value	4.661		
Average yield loss	22.951		
% yield loss	29.787		
CD(0.05)	1.064		

*Treated:(*Paecilomyces lilacinus*@ 2kg a.i/ha)

Table 5. Effect of *Paecilomyces lilacinus* treatment on number of root galls/egg masses gall index and Rf(reproduction factor) of root-knot nematode, *M. incognita* infesting Cucumber, Banki Local

Replications	Number of root galls/egg masses/plant at termination			Gall index/plant at termination			Rf (reproduction factor)		
	Treated	Untreated Control	% decrease over control	Treated	Untreated control	% decrease over control	Treated	untreated control	% decrease over control
1	47	54		3.8	5		0.57	1.19	
2	38	43		3.3	5		0.39	1.43	
3	34	40		3.2	5		0.36	1.14	
4	49	51		4	5		0.61	1.18	
5	35	42		3.4	5		0.53	1.11	
6	44	52		3.7	5		0.49	1.10	
7	47	51		3.9	5		0.64	1.19	
8	45	54		3.3	5		0.43	1.23	
9	37	44		3.6	5		0.60	1.05	
10	48	56		4	5		0.86	1.17	
SE(m)±	0.667			0.09			0.06		
t value	9.438			14.31			9.87		
CD(0.05)	7.484			1.82			0.83		

* **Rf (reproduction factor)=final population/initial population**

* **Treated: (*Paecilomyces lilacinus*@ 2kg a.i/ha)**

The present study that provides same basic information relating to host-pathogen interaction and bio-chemical mechanism of resistance. The above knowledge of physiological and bio-chemical events during the post-infection period have initiated search for molecules, which can trigger the function of enzymes involve in hyper sensitive response, production of phytoalexins lignin or other secondary metabolites which are detrimental to nematode feeding and development.

Antagonistic fungi, *Paecilomyces lilacinus* (Bio-nemton), is a biological insecticide based on a selective strain of naturally occurring entomopathogenic fungus, *Paecilomyces lilacinus*. *Paecilomyces lilacinus* produces proteases and chitinase enzymes that weakens nematodes so as to enable a narrow infection peg to be established and push through the insect's exterior and pathogenise it from within. The fungus is specific to Nematodes. Nematodes die before the fungus is visible.

Finally, the present investigation clearly indicated that *Meloidogyne incognita* played key role in altering the normal physiology and biochemical processes of the tested host plant. Further, it is opinion that the basic information provided in this investigation will certainly be helpful to understand the complicated areas of the biochemical mechanisms of plant nematode-interaction in relating to root-knot and other plant parasitic nematodes.

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