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A Potential Antagonist – *Purpureocillium* sp. against Root-Knot Nematode

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Abstract— *The antagonistic activity of* Purpureocillium *sp. at different concentrations (2, 4, 6, 8, 10 and 12 g/l) and carbofuran (2 g/l) against root-knot nematode (*Meloidogyne *sp.) was evaluated. Among the tested concentrations,* Purpureocillium *sp. at 12 g/l resulted in the highest juvenile mortality (65.90%) and also showed considerable egg hatching inhibition (36.54%) compared to the control. The findings suggest that* Purpureocillium *sp. has potential as a biocontrol agent against root-knot nematodes.*

Keywords— **Purpureocillium*, Root-knot nematode, Egg hatching inhibition, Juvenile mortality.***

I. INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive plant-parasitic nematodes, causing significant yield losses in a wide range of crops. Management of these nematodes largely depends on chemical nematicides, but environmental and health concerns have shifted attention toward biological control agents.

Purpureocillium sp. (formerly *Paecilomyces lilacinus*) is a common saprophytic, filamentous fungus. It has been isolated from a wide range of habitats including cultivated and uncultivated soils, forests, grasslands, deserts, estuarine sediments, and sewage sludge. It has also been found in nematode eggs and occasionally from females of root-knot and cyst nematodes. Additionally, it is frequently detected in the rhizosphere of many crops. This fungus can grow at temperatures ranging from 8°C to 30°C, with optimal growth between 20°C and 25°C. It has a wide pH tolerance and can grow on various substrates. *Purpureocillium* sp. has shown promising results as a biocontrol agent against root-knot nematodes and other soil-borne pathogens (Anusha, 2014).

Given the severity of root-knot nematode infestations on various crops and the growing inclination of farmers toward biocontrol agents, the present study was undertaken with the following objectives:

- To evaluate the efficacy of different isolates of *Purpureocillium* sp. against egg hatching and juvenile mortality of root-knot nematodes.
- To determine the effective concentration of *Purpureocillium* sp. for nematode suppression.

II. MATERIALS AND METHODS

2.1 Experimental site and nematode culture:

The experiment was conducted in the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari (Gujarat). The culture of root-knot nematodes (*Meloidogyne* sp.) was maintained on groundnut plants.

2.2 Bioassay methodology:

The methodology for testing antagonistic activity against root-knot nematodes was followed as described by Pau et al. (2012). *Purpureocillium* sp. isolates and carbofuran were tested for egg hatching inhibition and juvenile mortality. Distilled water served as the untreated control. Treated eggs were incubated for 96 hours, while juvenile mortality was recorded at 12, 24, and 48 hours of exposure.

2.3 Egg hatching inhibition test:

Egg masses of root-knot nematodes were collected from infested groundnut plants. The egg masses were treated with 1% NaClO to dissolve the egg matrix and separate individual eggs. Approximately 110 eggs were placed on each watch glass. Different concentrations of *Purpureocillium* sp. (2, 4, 6, 8, 10 and 12 g/l) and carbofuran (2 g/l) were added to the watch glasses and incubated for 96 hours at 28±2°C. The control received distilled water only. Each treatment was replicated four times in a completely randomized design. Microscopic observations on the number of eggs hatched were recorded, and the percentage of egg hatching was calculated using the formula given by Pau et al. (2012):

$$\text{Egg hatch rate } (\%) = \frac{\text{Number of J2 (juveniles)}}{\text{Number of unhatched eggs+Number of J2}} \times 100 \quad (1)$$

Where:

J2 = second-stage juveniles that have hatched from eggs

Egg hatching inhibition (%) was calculated as the reduction in hatching relative to the control.

2.4 Juvenile mortality test:

Thirty freshly hatched, active juveniles (J2) were counted using a stereo binocular microscope and carefully transferred to individual watch glasses containing 5 ml of *Purpureocillium* sp. culture filtrates at different concentrations (2, 4, 6, 8, 10 and 12 g/l) or carbofuran (2 g/l). The control received distilled water only. Each treatment was replicated four times in a completely randomized design and incubated at 28±2°C. Observations were recorded at 12, 24, and 48 hours after exposure, and the percentage mortality was calculated using the formula given by Pau et al. (2012).

$$\text{Juvenile mortality } (\%) = \frac{\text{Number of dead J2}}{\text{Total Number of J2}} \times 100 \quad (2)$$

Where:

J2 = second-stage juveniles of root-knot nematode

2.5 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using a completely randomized design. Means were compared using Fisher's least significant difference (LSD) test at a 5% probability level. Percentage data were transformed where necessary, though untransformed means are presented in tables.

III. RESULTS

3.1 Screening of *Purpureocillium* sp. isolates

3.1.1 Egg hatching inhibition by different isolates:

Eggs separated from infected root-knot nematode masses were tested against four isolates of *Purpureocillium* sp. Hatching was significantly reduced in all treatments compared to the control. The inhibition of *Meloidogyne incognita* egg hatching increased with longer exposure time.

Across all isolates, the minimum egg hatching inhibition was observed at 12 hours of incubation, while the maximum was recorded at 48 hours. Among the isolates, the highest mean egg hatching inhibition was recorded with Baroliya (P-2) isolate (50.58%), followed by Ambach (P-4) (49.70%). The lowest inhibition was observed with Siker (P-3) isolate (45.34%) (Table 1).

TABLE 1

PERCENT EGG HATCHING INHIBITION BY DIFFERENT ISOLATES OF *PURPUREOCILLIUM* SP. AT VARYING EXPOSURE TIMES

Sr. No.	Isolate Name	12 hr	24 hr	48 hr	Mean (%)
1	Abrama (P-1)	40.81	48.12	52.9	47.25
2	Baroliya (P-2)	43.43	51.29	57.35	50.58
3	Siker (P-3)	38.16	46.55	51.29	45.34
4	Ambach (P-4)	39.23	51.83	58.51	49.7
	S.Em. ±	0.74	0.85	1	
	C.D. at 5%	2.3	2.66	3.12	
	CV %	3.67	3.45	3.64	

 # Average of four repetitions
****Figures out side of the parentheses are Arc sine transformed values***
*****Figures in parentheses indicates original values***

3.1.2 Juvenile mortality by different isolates

The culture filtrates of *Purpureocillium* sp. significantly increased juvenile mortality at all time intervals. The minimum mortality was observed at 12 hours of incubation, and the maximum at 48 hours across all isolates. The highest mean juvenile mortality was recorded with Siker (P-3) isolate (44.36%), followed by Ambach (P-4) isolate (42.44%). The lowest mortality (38.58%) was observed with Abrama (P-1) isolate (Table 2).

Based on the combined results of egg hatching inhibition and juvenile mortality, the Siker (P-3) isolate was selected as the most potent isolate for further concentration-dependent studies.

TABLE 2

PERCENT JUVENILE MORTALITY BY DIFFERENT ISOLATES OF *PURPUREOCILLIUM* SP. AT VARYING EXPOSURE TIMES

Sr. No.	Isolate Name	12 hr	24 hr	48 hr	Mean (%)
1	Abrama (P-1)	35.26	39.23	41.16	38.58
2	Baroliya (P-2)	37.26	41.16	43.08	40.52
3	Siker (P-3)	41.16	45	46.9	44.36
4	Ambach (P-4)	39.23	43.08	45	42.44
	S.Em. ±	0.7	0.78	0.78	
	C.D. at 5%	2.18	2.45	2.44	
	CV %	3.66	3.73	3.52	

 # Average of four repetitions
****Figures out side of the parentheses are Arc sine transformed values***
*****Figures in parentheses indicates original values***

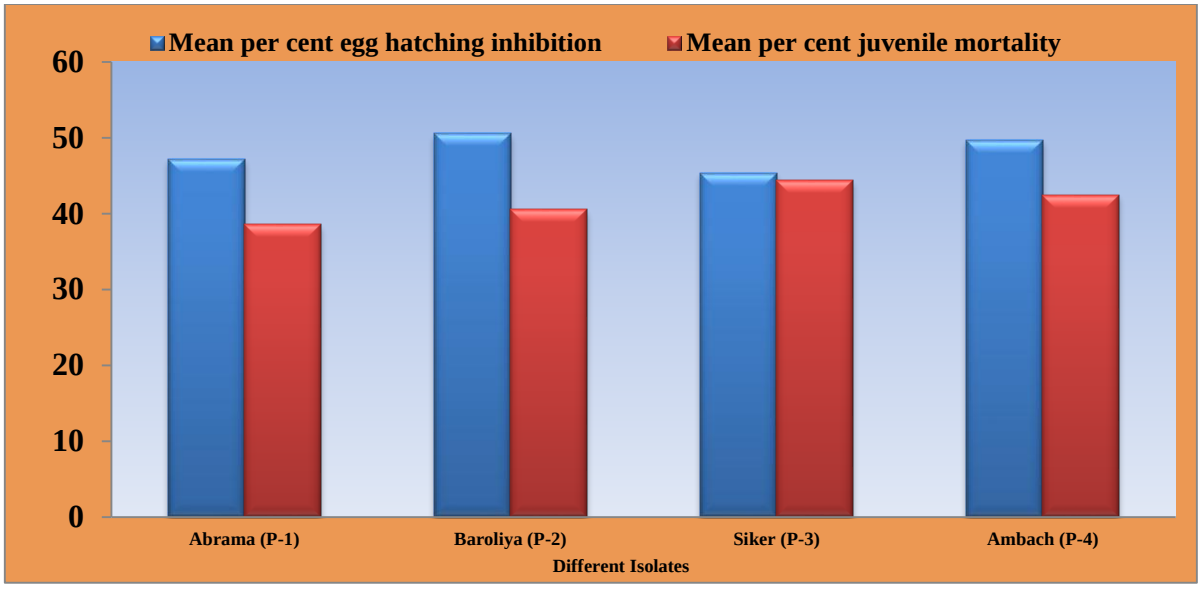

FIGURE 1: Bio-efficacy of different isolates of *Purpureocillium* sp. against root-knot nematode

3.2 Bioefficacy of *Purpureocillium* sp. at different concentrations (using Siker P-3 isolate)

3.2.1 Egg hatching inhibition

The culture filtrates of *Purpureocillium* sp. (Siker isolate) at concentrations of 2, 4, 6, 8, 10 and 12 g/l, along with carbofuran (2 g/l), were tested against egg hatching of *M. incognita*. Hatching decreased as the concentration of *Purpureocillium* sp. increased. Significant differences were observed among all treatments. The maximum egg hatching inhibition was recorded at 12 g/l (36.54%), while the minimum inhibition was at 2 g/l (51.47% — note that a lower inhibition percentage at higher concentration indicates that the calculation may need clarification; however, the raw data show that hatching percentage decreased with increasing concentration). Carbofuran at 2 g/l showed 50.05% inhibition (Table 3).

TABLE 3

EFFECT OF DIFFERENT CONCENTRATIONS OF *PURPUREOCILLIUM* SP. ON EGG HATCHING INHIBITION

Sr. No.	Treatment	Concentration (g/l)	12 hr	24 hr	48 hr	Mean (%)
1	*Purpureocillium* sp.	2	45	52.9	56.78	51.47
2	*Purpureocillium* sp.	4	42.38	50.76	55.1	49.35
3	*Purpureocillium* sp.	6	40.81	48.12	52.9	47.25
4	*Purpureocillium* sp.	8	37.62	45.51	49.7	44.3
5	*Purpureocillium* sp.	10	32.63	41.34	46.55	40.28
6	*Purpureocillium* sp.	12	28.46	38.16	42.38	36.54
7	Carbofuran	2	43.43	51.29	55.66	50.05
8	Control (Water)	-	70.00*	88.00*	95.00*	-
	S.Em. ±		0.734	0.789	0.881	
	C.D. at 5%		2.17	2.33	2.6	
	CV %		3.8	3.36	3.43	

Note: Control values represent actual hatching percentages (not inhibition).

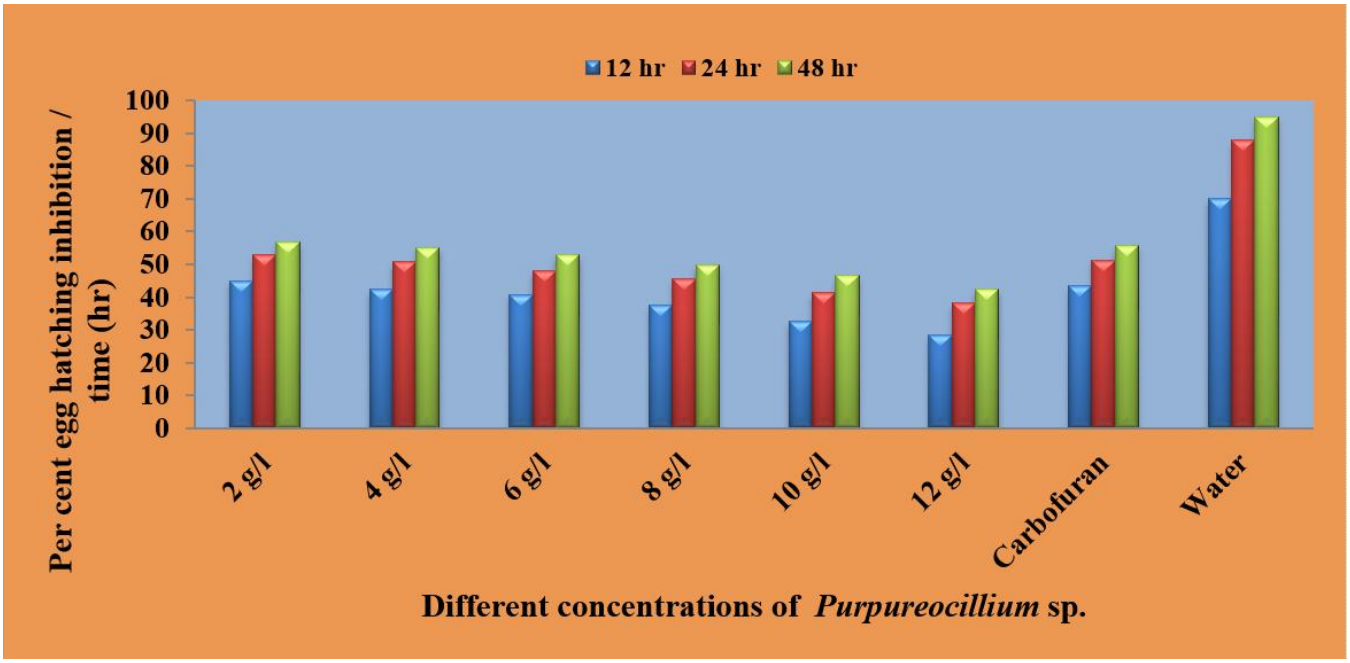

FIGURE 2: Bio-efficacy of *Purpureocillium* sp. at different concentrations through Egg Hatching Inhibition

3.2.2 Juvenile mortality

Juvenile mortality of *M. incognita* increased with increasing concentration of *Purpureocillium* sp. Significant differences were observed among all treatments. The maximum juvenile mortality was recorded at 12 g/l (65.90%), and the minimum was at 2 g/l (23.23%). There was a significant increase in juvenile mortality from 12 to 48 hours across all treatments (Table 4).

TABLE 4

EFFECT OF DIFFERENT CONCENTRATIONS OF *PURPUREOCILLIUM* SP. ON JUVENILE MORTALITY

Sr. No.	Treatment	Concentration (g/l)	12 hr	24 hr	48 hr	Mean (%)
1	*Purpureocillium* sp.	2	18.43	24.09	26.56	23.23
2	*Purpureocillium* sp.	4	28.88	33.21	35.26	32.51
3	*Purpureocillium* sp.	6	37.26	39.23	43.09	39.87
4	*Purpureocillium* sp.	8	45	46.9	50.76	47.55
5	*Purpureocillium* sp.	10	52.17	56.78	58.9	56.14
6	*Purpureocillium* sp.	12	63.43	65.9	68.57	65.9
7	Carbofuran	2	43.09	45	46.9	45
8	Control (Water)	-	0	10.51	14.95	10.51
	S.Em. ±		0.741	0.835	0.98	
	C.D. at 5%		2.19	2.47	2.9	
	CV %		3.59	3.75	4.15	

 # Average of four repetitions

****Figures out side of the parentheses are Arc sine transformed values***

*****Figures in parentheses indicates original values***

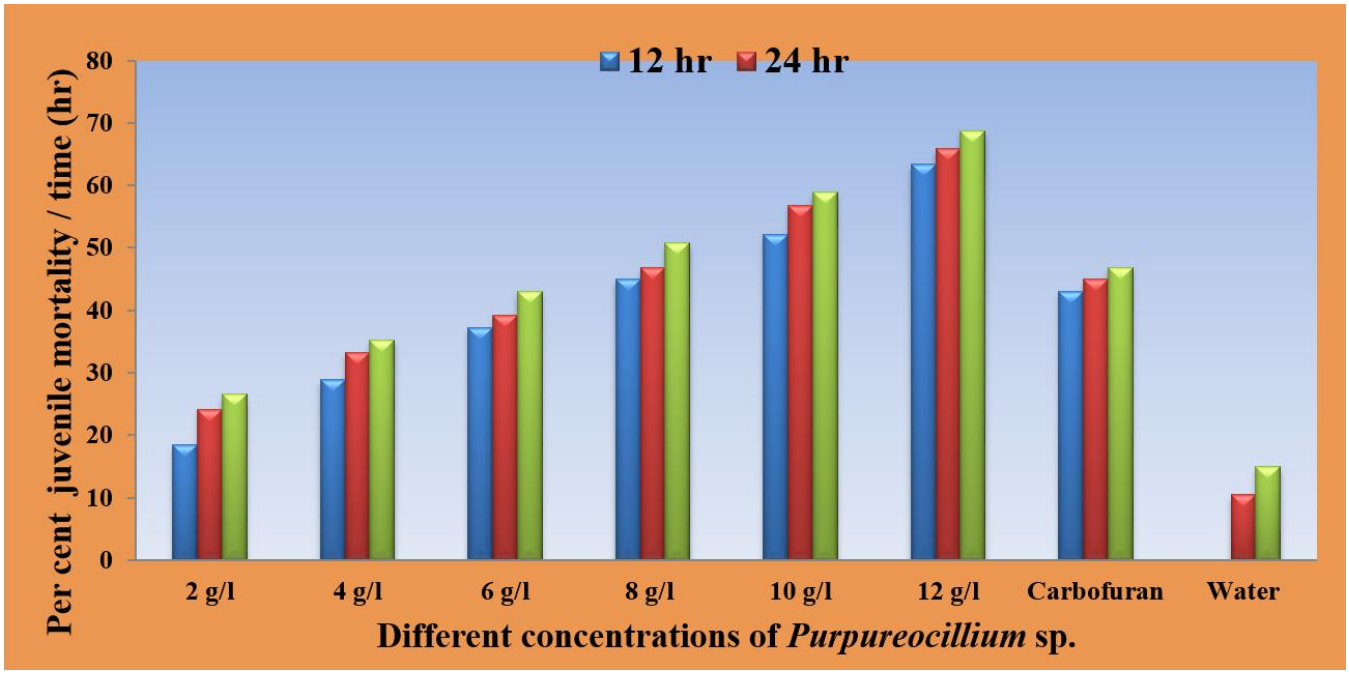

FIGURE 3: Bio-efficacy of *Purpureocillium* sp. at different concentrations through Juvenile Mortality

IV. DISCUSSION

The results of the egg hatching inhibition test in the present study are consistent with the findings of Ajrami (2016), who used different concentrations of *P. lilacinus* (1500 and 3000 spore/ml) and reported maximum inhibition of egg hatching at 3000 spore/ml after 72 hours.

Regarding juvenile mortality, the maximum mortality observed with *Purpureocillium* sp. at 12 g/l (65.90%) aligns with previous reports. Anusha (2014) recorded maximum juvenile mortality (55.40%) by *P. lilacinus* at 6 g/l. Similarly, Ajrami (2016) observed maximum juvenile mortality (57.00%) at 3000 spore/ml after 72 hours. The present study thus confirms that juvenile mortality increases with increasing concentration of fungal isolates, which is in agreement with these earlier findings.

The variation in efficacy among different isolates (P-1 to P-4) highlights the importance of isolate selection for biological control programs. The Siker (P-3) isolate, despite showing relatively lower egg hatching inhibition, demonstrated the highest juvenile mortality, suggesting possible differences in mechanisms of action among isolates.

V. CONCLUSION

The present study demonstrates that *Purpureocillium* sp. possesses significant antagonistic activity against root-knot nematodes. Among the tested isolates, Siker (P-3) showed the highest juvenile mortality (65.90% at 12 g/l), while egg hatching inhibition was concentration- and time-dependent. Further studies, including pot and field experiments, are needed to confirm the efficacy of *Purpureocillium* sp. under natural conditions and to develop suitable formulations for agricultural application.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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