Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7 (3): 456-460

Received: 19-03-2019
Accepted: 23-04-2019

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Colonization pattern of nematode egg parasitic fungus, *Pochonia chlamydosporia* (TNAU Pc-001) in soil

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Abstract
Nematode egg parasitic fungus, *Pochonia chlamydosporia* (TNAU Pc001) is widely utilized as a biocontrol agent in nematode management. *P. chlamydosporia* is endophytic in nature but also lives saprophytically in the soil when the host nematode is absent. Therefore the present study was undertaken to investigate *P. chlamydosporia* colonization in different soil type and various depths. An experiment was conducted to identify colonization pattern in different types of soil. Pot mixture (Red soil, Sand, FYM at 2:1:1) served as control. The results revealed that clay (Cotton) soil showed highest colonization (6.53×10^7 cfu/g). Another set of experiment was conducted to assess the depth of colonization in different soils in test tubes and samples were plated from different depth (2 cm, 4 cm and 6 cm). The results indicated that the CFU was found higher in clay (Rice) soil (5.78×10^6 cfu/g) at 6 cm depth compared to control. Colonization was found up 6 cm in all type of soil.

Keywords: Nematode egg parasitic fungus, *Pochonia chlamydosporia*, colonization, Soil type, depth

1. Introduction
Plant parasitic nematodes are one of the major limiting factors in crop productivity. Biological control is considered to be an eco-friendly management technique. Among various bioagents used, *Pochonia chlamydosporia* is one of the most promising nematophagus fugus. *Pochonia chlamydosporia* (Goddard) Zare and Gams.W, 2001 [12] is a potential nematode egg parasitic fungus (Wilcox and Tribe, 1974) [11]. This fungus infests the eggs and females of economically important species of plant parasitic nematodes such as root knot nematode *Meloidogyne* sp. (Hidalgo-diaz *et al.*, 2000) [3], cyst nematodes *Globodera sp.* and *Heterodera sp.* (Kerry and Crump, 1977) [1]. In the absence of host, the fungus remains in the soil as saprophyte.
In Tamil Nadu, it is widely being used for the management of root knot, reniform and citrus nematodes by vegetable and fruit growing farmers. The mode of action of *P. chlamydosporia* (TNAU Pc-001) was documented by Swarnakumari and Kalaiarasan (2017) [10]. It produces chlamydospores in the absence of host nematode. Chlamydospores are resistant to temperature, pH and moisture regimes. The endophytic nature of *P. chlamydosporia* has been proved in some Graminae and Solanaceae crop species (Lopez-Lorca *et al.*, 2008) [5]. It can also survive in weed plant roots as endophyte (Pavlos Bouchagier, 2018) [8]. Since, *P. chlamydosporia* is endophytic in nature; they may parasitize the eggs as well as nematodes present inside the roots. Research works on ecology of *P. chlamydosporia* has been carried out in other countries. But in India, only minimum research work has been done to understand the colonization pattern and conidial transmission in soil. Hence *in vitro* studies were carried out to document the growth of *P. chlamydosporia* in different soils by recording vertical and horizontal colonization of mycelia. These basic studies are essential to formulate the precise management strategies for different crops.
The colonization pattern of *P. chlamydosporia* (Isolate TNAU Pc-001) in different types of soil both vertically and horizontally is described in this research paper.

2. Materials and Methods
2.1 *Pochonia chlamydosporia* (TNAU Pc-001) fungus culture
The culture of nematode egg parasitic fungus, *Pochonia chlamydosporia* (TNAU Pc-001) was obtained from the Department of Nematology,

Tamil Nadu Agricultural University, Coimbatore. The culture was sub-cultured on Potato Dextrose agar (PDA: Potato- 250 g; Dextrose- 20 g; Agar- 20 g; Sterilized distilled water- 1000 ml) medium and incubated at 25°C temperature in an incubator (Genuine BOD Incubator) for 10days.

2.2 Soil samples from five different cropping system

Soil samples collected from five different cropping systems in various locations, *viz*., Rice (Paiyur), Guava (Paiyur), Millets (Coimbatore), Cotton (Coimbatore), Rice (Coimbatore). Soil samples were subjected to texture analysis (Table -1), nutrient analysis, pH, EC and colour (Table -2).

Table 1: Texture of the collected soil sample

Treatments	Silt	Clay	Sand	Texture	Colour
Sample -1	7.50	10.90	80.70	Sandy soil	Brown
Sample -2	20.00	28.40	67.40	Sandy clay loam soil	Red
Sample -3	22.50	13.90	63.60	Sandy loam soil	Red
Sample -4	7.50	43.40	24.55	Clay soil	Brown
Sample -5	10.00	45.90	31.25	Clay soil	Black

Table 2: Nutrient status of the collected soil sample

Treatments	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)	pH	EC	Lime status
Sandy soil (T1)	171.00	28.0	176	7.44	0.16	Calcareous
Sandy clay loam soil (T2)	179	36.0	534	7.40	0.18	Non Calcareous
Sandy loam soil (T3)	118	10.0	222	7.55	0.14	Calcareous
Clay soil- Cotton (T4)	193	25.0	765	7.76	0.28	Calcareous
Clay soil- Rice (T5)	210	22.0	588	7.75	1.67	Calcareous
Pot mixture (T6)	319	195.0	628	6.67	0.41	Non Calcareous

2.3 Colonization pattern of *P. chlamydosporia* in different type of soil

The soil was collected from different cropping system, shade dried, sieved (20 mesh) and mixed with vermicompost. Then the sterilised soil was filled in Petri plates (9 cm). A ring was inserted into the soil and 5 ml sterile distilled water was added. A nylon mesh placed with 8 mm diameter fungal disc of *P. chlamydosporia* placed over the ring (Figure.1). This setup was incubated for 15 days at 25°C temperature in an incubator (Genuine BOD Incubator). Then the colony forming units (CFU) were determined using serial dilution technique with Potato Dextrose Agar (PDA) as culture media.

The above mentioned procedure was followed for the five different types of soil viz., sandy soil, sandy clay loam soil, sandy loam soil, clay (Cotton) soil, clay (Rice) soil. A control was maintained with pot mixture. Treatments- 6; Replication- 4; Statistical design adopted- Complete Randomized Design (CRD)

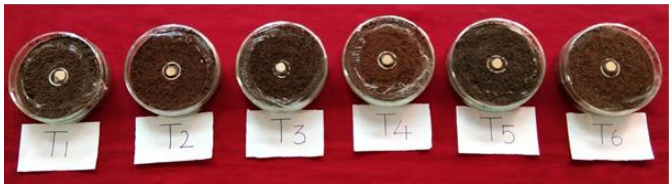

Fig 1: Colonization efficiency of *P. chlamydosporia* in different type of soil

T1- Sandy soil; T2- Sandy clay loam soil; T3- Sandy loam soil; T4- Clay (Cotton) soil; T5- Clay (Rice) soil; T6- Pot mixture

2.4 Colonization pattern of *P. chlamydosporia* in different type of soil at different depths

The soil was collected from different cropping system, shade dried, sieved (20 mesh) and mixed with vermicompost. Then the sterilised soil was filled in test tubes (13 cm long) and 5 ml sterile distilled water was added. A nylon mesh placed with 8 mm diameter fungal disc of *P. chlamydosporia* placed above the soil in test tubes (Figure.2). This setup was incubated for 15 days at 25°C temperature in an incubator (Genuine BOD Incubator). Then the colony forming units (CFU) were determined using serial dilution technique at different depths (2cm, 4cm and 6cm) with PDA as culture media.

The above mentioned procedure was followed for the five different types of soil viz., sandy soil, sandy clay loam soil, sandy loam soil, clay (Cotton) soil, clay (Rice) soil. A control was maintained with pot mixture pot mixture. Treatments- 6; Replication- 4; Statistical design adopted- CRD.

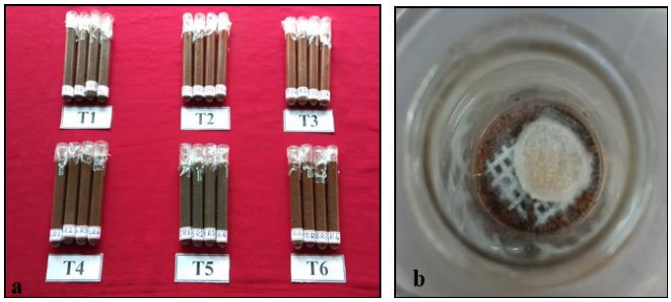

Fig 2: Colonization efficiency of *P. chlamydosporia* at different depth

a. T1- Sandy soil; T2- Sandy clay loam soil; T3- Sandy loam soil; T4- Clay (Cotton) soil; T5- Clay (Rice) soil; T6- Pot mixture **b**. *P. chlamydosporia* fungal disc placed over the mesh inside the test tube

2.4 Statistical analysis

All data were subjected to statistical analysis of variance (ANOVA). The data were tested transformed by log transformation before being subjected to ANOVA. Means were separated using the Duncan's multiple range test (DMRT) test at P≤0.01.

3. Results

3.1 Colonization pattern of *P. chlamydosporia* in different type of soil

The colonization of *P. chlamydosporia* in different type of soil was assessed after 15 days of incubation (Figure.3). CFU data from the five type of soil and pot mixture (control) were analysed to assess the efficacy of *P. chlamydosporia*

colonization and abundance in different texture of soil (Figure.4). The results showed that highest CFU load (6.53×10^7 cfu/g) was found in clay (cotton) soil compared to the control. There was a significant difference between the treatments in CFU mean (Table -3).

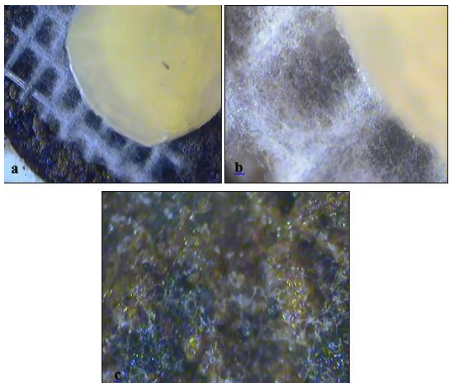

Fig 3: Efficient colonization of *P. chlamydosporia* n soil
a & **b.** Mycelial growth in wire mesh **c**. Fungal mycelia growth in soil

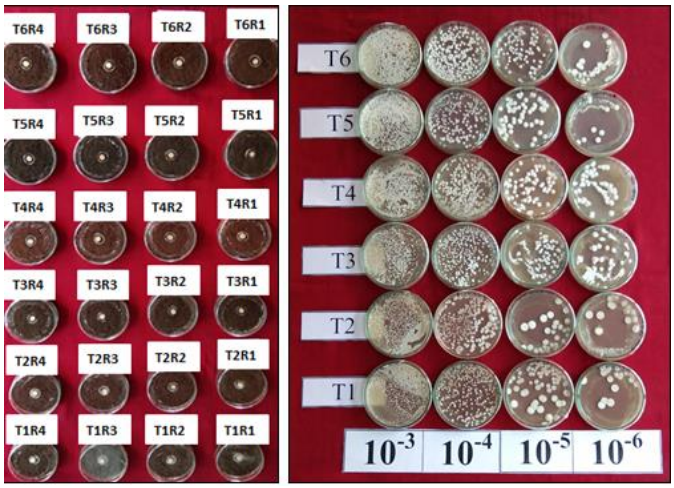

Fig 4: CFU load of *P. chlamydosporia* in different type of soil at different dilution
T1- Sandy soil; T2- Sandy clay loam soil; T3- Sandy loam soil; T4- Clay (Cotton) soil; T5- Clay (Rice) soil; T6- Pot mixture

Table 3: Colonization of *P. chlamydosporia* in different type of soil (CFU)

Treatments/ Dilution	10^{-3}	10^{-4}	10^{-5}	10^{-6}
T1- Sandy soil	1177.50^{bc} (3.07)	377^{ab} (2.58)	70.75^{a} (1.85)	46^{a} (1.66)
T2- Sandy clay loam soil	1132.25^{bc} (3.05)	318.25^{ab} (2.50)	61.75^{a} (1.79)	25^{b} (1.40)
T3- Sandy loam soil	$1014b^{c}$ (3.00)	331^{ab} (2.52)	74.25^{a} (1.87)	51.25^{a} (1.71)
T4- Clay (Cotton) soil	1634^{a} (3.21)	410.5^{ab} (2.61)	82.75^{a} (1.92)	65.25^{a} (1.81)
T5- Clay (Rice) soil	1312^{ab} (3.11)	286.25^{b} (2.46)	56^{ab} (1.75)	49.25^{a} (1.69)
T6- Pot mixture	966^{c} (2.98)	199.25^{c} (2.30)	41.25^{b} (1.62)	21.5^{b} (1.33)
SEd	0.0640	0.0737	0.0979	0.1056
CD (p=0.01)	0.1842	0.2121	0.2819	0.3040
CV (%)	2.95	4.19	7.79	9.45
In the columns the numbers followed by alphabets are grouped based on DMRT				

3.2 Colonization pattern of *P. chlamydosporia* in different type of soil at different depths

In this experiment the colonization of *P. chlamydosporia* in different type of soil at different depths were assessed after 15 days of incubation. CFU data from the five type of soil and pot mixture (control) at different depth *viz*., 2 cm, 4 cm and 6 cm were analysed to assess the colonization of *P. chlamydosporia* and abundance in different depth of soil with various texture (Figure.5). The fungus was able to colonize up to 6 cm in all type of soil and also identified that the CFU was stable in clay (Rice) soil at different depth (6 cm depth- 5.78×10^6 cfu/g). There was a significant difference in colonization between the treatments (Table-4).

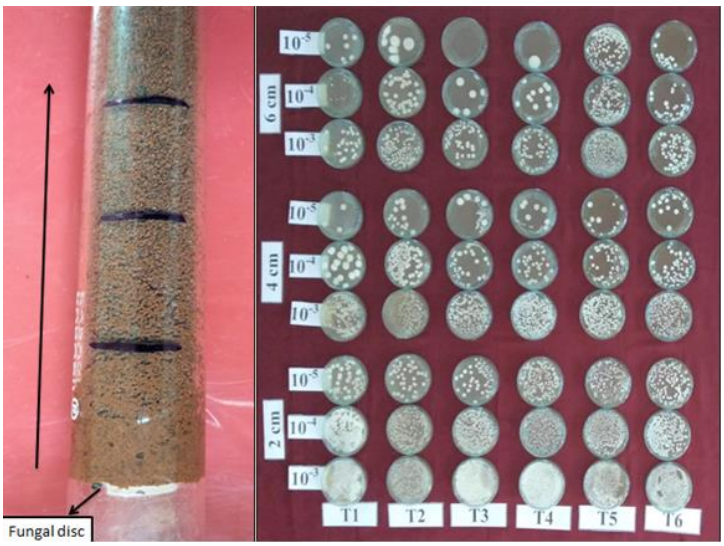

Fig 5: CFU load of *P. chlamydosporia* at different depth in different type of soil
a. Different depth in test tube b. CFU at different depth in different dilution

Table 4: Colony forming units (CFU) in different type of soil at different depth

Treatments/ Dilution	2 cm			4cm			6 cm		
	10^{-3}	10^{-4}	10^{-5}	10^{-3}	10^{-4}	10^{-5}	10^{-3}	10^{-4}	10^{-5}
T1-Sandy soil	1270.75^{b} (3.10)	132.25^{b} (2.12)	71.75^{b} (1.86)	154.00^{b} (2.19)	46.75^{bc} (1.67)	5.75^{c} (0.76)	64.75^{b} (1.81)	23.00^{b} (1.36)	9.75^{c} (0.99)
T2-Sandy clay loam soil	1797.00^{a} (3.25)	479.25^{a} (2.68)	92.50^{ab} (1.97)	326.25^{a} (2.51)	50.75^{bc} (1.71)	13.25b^{c} (1.12)	135.00^{a} (2.13)	21.75^{b} (1.34)	9.25^{c} (0.97)
T3-Sandy loam soil	1979.00^{a} (3.30)	521.25^{a} (2.72)	101.75^{ab} (2.01)	196.75^{ab} (2.29)	21.75^{d} (1.34)	3.00^{d} (0.48)	56.00^{b} (1.75)	4.50^{c} (0.65)	0.50^{d}-
T4-Clay (Cotton) soil	1815.00^{a} (3.26)	506.50^{a} (2.70)	147.25^{ab} (2.17)	334.25^{a} (2.52)	40.50^{c} (1.61)	14.25^{b} (1.15)	61.75^{b} (1.79)	13.25^{b} (1.12)	0.75^{d}-
T5-Clay (Rice) soil	2042.00^{a} (3.31)	545.00^{a} (2.74)	156.25^{a} (2.19)	380.25^{a} (2.58)	93.00^{a} (1.97)	35.75^{a}b (1.55)	152.00^{a} (2.18)	71.50^{a} (1.85)	57.75^{a} (1.76)
T6-Pot mixture	1382.50^{b} (3.14)	385.75^{a} (2.59)	137.25^{a} (2.14)	318.50^{a} (2.50)	71.00^{ab} (1.85)	29.25^{a} (1.47)	91.00^{ab} (1.96)	25.00^{b} (1.40)	22.00^{b} (1.34)
SEd	0.0399	0.1447	0.1143	0.1264	0.0899	0.1652	0.1311	0.1708	0.1280
CD (p=0.01)	0.1150	0.4167	0.3291	0.3639	0.2588	0.4755	0.3774	0.4919	0.3686
CV (%)	1.75	8.03	7.96	7.47	7.58	22.45	9.67	19.45	24.37
In the columns the numbers followed by alphabets are grouped based on DMRT									

4. Discussion

P. chlamydosporia is a potential egg parasitic fungus. Study on ecology of the fungus is required to know the parasitization of nematode eggs and biocontrol efficiency. The fungus requires moisture content for mycelium growth and sporulation. This is also in accordance with Lindenfelser and Ciegler (1975) [4]. The results revealed the colonization of fungal mycelium was high in clay textured soil compared to other types. This may be due to the higher water holding capacity of the soil and impact of water over fungal growth. The duration for sporulation and production cycle is influenced by the proportion of water content in the media (Nagesh *et al*., 2007) [6]. The experiment was conducted by Monteiro *et al*. (2019) [7] to manage nematode revealed that the colonization of *P. chlamydosporia* was higher in sandy soil than in clay soil when applied as seed treatment after 50 days.

But in contrast, the observation showed that the colonization of *P. chlamydosporia* was higher in clay textured soil after 15 days of incubation. This difference may be due to the impact of initial water content and incubation period. The current study showed that the fungus was able to colonize up to 6 cm vertically in different type of soil. This is also in accordance with the findings of Monteiro *et al*. (2019) [7]. The present investigation confirmed that the colonization of *P. chlamydosporia* was higher in clay textured soil due to the higher amount of nitrogen and potassium content. The results of current work showed that *P. chlamydosporia* grows efficiently in sterilised soil which is in agreement with the study conducted by the Siddiqui *et al*. (2009) [9]. They have also reported that there was no significant difference in the growth of *P. chlamydosporia* in soils of different texture while the results of current study confirms that there is a significant difference in growth of this fungus related to the soil texture. Addition of vermicompost to soil might have resulted in higher colonization of the fungus in the present study. This result is supported by the work conducted by de-Leij *et al*. (1993) [2] who state that the *P. chlamydosporia* proliferation was higher in organic soil than the mineral soils.

5. Conclusion

The nematode egg parasitic fungus, *P. chlamydosporia* colonised efficiently in clay (Cotton) soil (6.53 $\times 10^{7}$ cfu/g) and depth of colonization was also higher in clay (Rice) soil (6 cm –5.78 $\times$ 10^{6} cfu/g). From this study it has been concluded that the fungus was able to colonize the soil upto 6 cm depth, so it may even colonize the soil beyond the 6 cm. Furthermore analysis on maximum colonization depth of *P. chlamydosporia* in soil has to be carried for understanding the behaviour of the fungus and its colonization ability on nematodes at higher depths. Hence it can also be used for deep rooted crops.

6. Acknowledgement

I delivered my sincere gratitude to the Department of Nematology in Tamil Nadu Agricultural University, Coimbatore for providing the culture of *P. chlamydosporia* and laboratory facilities to conduct the experiment successfully.

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