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Studies on compatibility of egg parasitic fungi with other biocontrol agents and carbofuran under *in vitro*

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Abstract
The egg parasitic fungi *viz*., *Pochonia chlamydosporia* and *E. aranearum* were studied for their compatibility with other biocontrol agents *viz*., *Pseudomonas fluorescens, Bacillus subtilis, Trichoderma viride, Paecilomyces lilacinus* and carbofuran under *in vitro*. The experiments were incubated at 28 ± 2 °C, the observations were recorded in 5 and 10 days intervals. The growth of *T. viride* and *P. lilacinus* was rapid when compared to *P. chlamydosporia* and *E. aranearum* isolates. Since both these fungi were compatible with each other, zone of inhibition was not observed. There was no inhibition in the growth of the fungus or bacterium in the medium. Growth of the fungal isolates was faster than *P. fluorescens* and *Bacillus subtilis*. Spore production in both the fungal isolate was not suppressed by *P. fluorescens* and *B. subtilis*. Carbofuran did not suppress the growth the fungus. The egg parasitic fungi, *P. chlamydosporia* and *E. arenearum* was found compatible with other biocontrol agents and carbofuran *in vitro*.

Keywords: *Pochonia chlamydosporia, E. aranearum,* biocontrol agents, carbofuran and *Meloidogyne incognita*

1. Introduction
The root knot nematode, *Meloidogyne incognita* is one of the major constraints in the production of vegetables (Stirling, 1991) [1]. The egg parasitic fungi, *Pochonia chlamydosporia* and *P. lilacinus* have been associated with soils which suppress the multiplication of cyst nematode populations (Kerry *et al.,* 1993) [2]. Nematophagous fungi are potential biological control agents of plant-parasitic nematodes. These are mostly facultative parasites and have the capability to infect mainly nematode eggs and juveniles (Jansson & Lopez-Llorca, 2004) [3]. In order to provide effective control of nematode multiplication,
P. chlamydosporia must establish in the soil and rhizosphere and survive, even in the absence of nematode hosts, and infect, parasitize and consume nematode eggs when they are produced on roots (Kerry and Jaffee, 1997) [4]. The egg parasitic fungi can colonize nematode reproductive structures and affect them. The sedentary stages of *Heterodera* and *Meloidogyne* are vulnerable to attack by these fungi either within the roots or on the root surface or in the soil. Once in contact with cysts and egg masses, these fungi grow rapidly and eventually parasitize all eggs in early embryonic developmental stages. The egg parasitic fungus, *Engyodontium aranearum* (Patent application No. 399/CHE/2012) to parasitized the potato cyst nematodes effectively; it is the first report from The Nilgiris (Muthulakshmi, 2011) [5]. Hence, the present investigation was aimed to study the compatibility of egg parasitic fungi with other biocontrol agents and chemical nematicide under *in vitro*.

2. Methodology
The compatibility of the egg parasitic fungi with other biocontrol agents *viz*., *Trichoderma viride*, *Paecilomyces lilacinus, Pseudomonas fluorescens* and *Bacillus subtilis*, and carbofuran were studied under laboratory conditions.

2.1 Egg parasitic fungi with *T. viride* and *P. lilacinus*
Compatibility of the biocontrol agents were tested by using dual culture technique (Dennis and Webster, 1971) [6]. A 9 mm diameter mycelial disc of 15 day old egg parasitic fungi was placed at one end and to the other end a disc of similar size from 4 days old culture of *T. viride* or *P. lilacinus* was placed in a Petri dish containing 15 ml of solidified PDA medium.

The plates were incubated at 28 ± 2 ºC. The compatibility of both the fungi was observed at 5 days after incubation (DAI).

2.2 Egg parasitic fungi with *P. fluorescens* and *B. subtilis*

To the Petri dish containing 15 ml of solidified PDA fungus disc of 9 mm was placed at one end and to the other end of the plate *P. fluorescens* or *B. subtilis* was streaked on the medium. The plates were incubated at 28 ± 2 ºC. The compatibility of both the fungus and bacterium was observed at 5 DAI.

2.3 Effects of carbofuran on egg parasitic fungi *in vitro*

A granular nematicide carbofuran was tested under laboratory condition. Potato dextrose agar was chosen for use in this experiment. Autoclaved PDA was cooled to room temperature before addition of the carbofuran. Carbofuran was added at 0.1 and 0.5 ppm, the recommended rate for application to the soil. The fungal disc was taken from 15 days old culture and transferred to Petri dish containing PDA and carbofuran. The observations were taken from 10 days after incubation (DAI).

3. Results and Discussion

3.1 Compatibility of *P. chlamydosporia* and *E. aranearum* with other biocontrol agents and carbofuran

The compatibility of the egg parasitic fungi, *P. chlamydosproia* and *E. aranearum* with other biocontrol agents *viz*., *T. viride P. lilacinus, P. fluorescens*, *B. subtilis* and carbofuran was studied so that they can be included in integrated nematode management practices. The growth of both the fungi (*P. chlamydosporia* and *E. aranearum*) was observed after 5 days of incubation in the medium. The growth of *T. viride* and *P. lilacinus* was rapid when compared to *P. chlamydosporia* and *E. aranearum* isolates. Since these both fungi not exhibiting any inhibition zone it is considered that both are compatible with each other (Table. 1 and Fig. 1, 2). As reported by Ahmad and Baker (1987) [7] the competitive saprophytic ability was higher for *T. viride* when compared to *P. lilacinus* which helps *T. viride* to cover the entire petri plate quickly when compared to *P. lilacinus.*

The present study also revealed that the growth of *P. fluorescens* and *B. subtilis*, *P. chlamydosporia* and *E. aranearum* isolates were observed at after 5 days of incubation. There was no inhibition in the growth of the fungus or bacterium in the medium, Growth of the fungal isolates was faster than *P. fluorescens* and *B. subtilis.* Spore production in both the fungal isolate was not suppressed by *P. fluorescens* and *B. subtilis* (Table. 1 and Fig. 1, 2) which is in accordance with Zaki and Mahmood (1993) [8] who reported that combined inoculation of *P. lilacinus* and *B. subtilis* to be the best for the control of root-rot disease complex in chickpea. Spore production and growth of *P. lilacinus* was also not arrested by *B. subtilis* and *P. lilacinus* was found to grow on the colonies of *B. subtilis*. Antibiotics and HCN produced by *P. fluorescens* (Freeman *et al*., 1975) [9] did not inhibit *P. chlamydosporia* and *E. aranearum.* In carbofuran amended medium the growth of *P. chlamydosporia* and *E. aranearum* isolates was observed after 5 days. Carbofuran did not suppress the growth the fungus (Table. 1 and Fig. 1, 2). Growth of *P. chlamydosporia* and *E. aranearum* was observed on the colonies of *P. fluorescens* similar to their compatibility with other biocontrol agents the egg parasitic fungi *P. chlamydosporia* and *E. aranearum* were found compatible with carbofuran under lab conditions.

Table 1: Compatibility of *P. chlamydosporia* and *E. aranearum* with other biocontrol agents and carbofuran

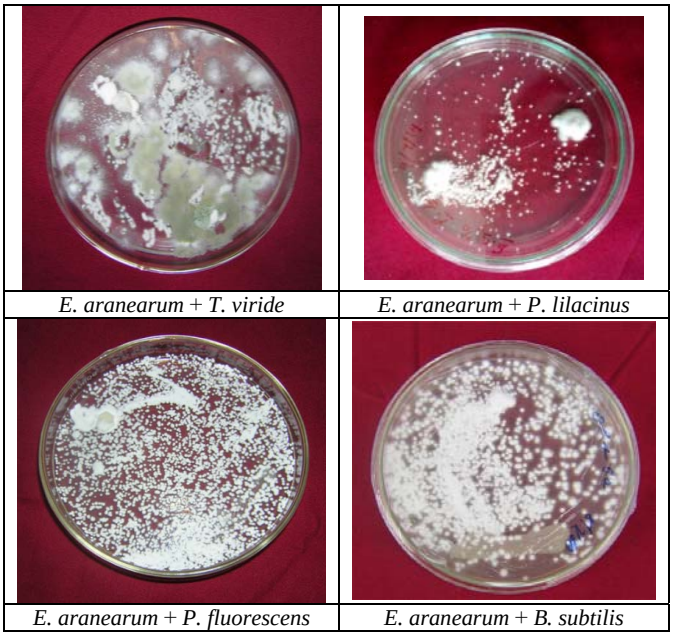
S. No.	Treatments	Compatibility
1.	*P. chlamydosporia* + *T. viride*	++
2.	*P. chlamydosporia* + P. *lilacinus*	++
3.	*P. chlamydosporia* + *P. fluorescens*	+++
4.	*P. chlamydosporia* + *B. subtilis*	+++
5.	*P. chlamydosporia* + Carbofuran	+++
6.	*E. aranearum* + *T. viride*	++
7.	*E. aranearum* + P. *lilacinus*	++
8.	*E. aranearum* + *P. fluorescens*	+++
9.	*E. aranearum* + *B. subtilis*	+++
10.	*E. aranearum* + Carbofuran	+++

+ less compatible
++ Moderately compatible
+++ highly compatible
- not compatible

P. chlamydosporia + *T. viride* | *P. chlamydosporia* + *P. fluorescens*

P. chlamydosporia + *B. subtilis* | *P. chlamydosporia* + Carbofuran

Fig 1: Compatibility of *P. chlamydosporia* with other biocontrol agents



E. aranearum + *T. viride* | *E. aranearum* + *P. lilacinus*

E. aranearum + *P. fluorescens* | *E. aranearum* + *B. subtilis*

E. aranearum + Carbofuran

Fig 2: Compatibility of *E. aranearum* with other biocontrol agents

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