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In Vitro Nematophagous Activity of Predatory Fungi on Infective Nematodes Larval Stage of Strongyloidae Family

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Abstract

AIM: The main goal of the present research conducted to assess the in vitro activity of the nematophagous fungi *Duddingtonia flagrans, Fusarium solani, Verticillium chlamidosporium,* and *Trichoderma harzianum.*

MATERIAL AND METHODS: Four isolates of fungi including *D. flagrans, F. solani* , *V. chlamidosporium* and *T. harzianum* were used in this study. Horse faeces were used to provide the larvae stage of Strongyloidae family for the experiments.

RESULTS: *D. flagrans* was the most effective fungus to reduce the population of the larval nematodes. *D. flagrans* was able to kill 100% of larvae after 14 days of incubation. The significant effect was seen after 7 days of incubation, therefore, the live larvae was decreased to 9, 11, 19 and 25 for *D. flagrans, V. chlamidosporium, F. solani* and *T. harzianum,* respectively.

CONCLUSION: Our results illustrated that *D. flagrans* were most successful fungus for reducing the number of *Strongylidae* family larva stage from horse faeces. Follow *D. flagrans,* the live larvae was significantly reduced for *V. chlamidosporium, F. solani* and *T. harzianum,* respectively.

Introduction

Many nematodes are parasites on plants, animals and humans and able to cause significant economic losses and serious health problems. The nematodes' eggs deposited in the environment via the faeces of their host, and survive for long time periods and creature an infection source for other animals and humans, and complete their biological cycle [1]. Gastrointestinal tract nematodes parasites of animals are the main issue that reduces the development of the livestock industry. Currently, the main technique for controlling nematodes population in livestock animals and field is chemotherapy against worm using banzimidasole carbamate drugs such as albendazole, mebendazole and cambendazole. However, in recent years there are many reports on resistance of nematodes against conventional anti parasites drugs and this method is expensive [2, 3]. Therefore, development of novel techniques is required to control of nematodes for the reason that the conventional methods are based on the use of nematicidal agents that are related to main environmental and health problems. Some control programs have been developed to reduce the undesirable effects of gastrointestinal parasitism. A promising strategy for controlling of parasitic nematodes is using natural enemies against these parasites [4-7]. A group of largely soil-living fungi have nematophagous activity. The quantity of nematode-trapping fungal species present in a particular soil and the population densities of them can significantly be diverse. The highest densities are typically found in autumn and 30 cm the upper of soil [8].

Several fungal species have been investigated as potential agents for biological control. In predator fungi group, the genera *Duddingtonia*, *Arthrobotrys* and *Monacrosporium* show up for their efficient environmental control of parasite nematodes [9-11].

One of the most well-known fungi is

Duddingtonia flagrans that are studied in several countries in the world [9, 11]. This fungus develops the three-dimensional nets and creates huge quantities of resistant spores. Chlamydospores increased the amount of fungus that passes throughout the gastrointestinal tract of sheep. Moreover, chlamydospores have the ability to germinate, make colonies in the faeces and destroy the fresh infective larvae and break off the life cycle of the parasite.

The main goal of the present research conducted to assess the in vitro activity of the nematophagous fungi *Duddingtonia flagrans, Fusarium solani, Verticillium chlamidosporium, and Trichoderma harzianum.*

Materials and Methods

Fungal strains

Four isolates of fungi included one from *D. flagrans , one from F. solani (*PTCC 5284)*, one from V. chlamidosporium (* PTCC 5179) *and one from T. harzianum (*IBRC-M 30059) *were incubated in* Petri dishes containing potato dextrose agar at 25°C for 10 days. After growth of the isolates, a culture disks, 4 mm in diameter, were transferred to 9 cm diameter. Petri dishes containing 20 mL of 2% water agar medium and were incubated for 10 days.

Preparation of Strongylidae Family Larval Stage

Horse faeces were used to provide the larvae stage for the experiments. The faeces were tested by light microscope to confirm that they were infected by egg parasite. The samples are incubated at room temperature for seven days to obtain third larvae stage. For collecting the larvae, Baermenn apparatus method was used. Subsequently, 20 grammes of faeces from infected horses was added on funnel containing sterile cloth and water and was incubated at room temperature for 24 hrs. Finally, the larvae were collected from the narrow side of the funnel. The collected larvae were rinsed 5 times with PBS solution and centrifuged for 2 min at 500 rpm and then for preventing the bacteria and fungi growth, penicillin-streptomycin (100 IU/ml) (Sigma, Germany) and amphotericin B (fungizone/Brstol-Myers Squibb, Paris) were added and kept at 4°C until use.

Co-culture of Fungi and Larvae

For removing the antibiotics, the larvae were washed with PBS solution for 10 times. One ml of larvae suspension containing 100 third stage nematode larvae from *Strongylidae* family was separately added to 2% water-agar medium Petri-dishes containing the fungal cultures. On days 1, 2, 3, 7, 14 and 21, the live larvae were counted by light microscope (10x) and recorded. One hundred larvae were added to water agar Petri dish without fungi as a control group. All experiments were repeated for three times.

Results

The results in Table 1 show that of all tested fungi, *D. flagrans* was the most effective fungus to reduce population of the larval nematodes.

Table1: The nematophagus effects of studied fungi on third stage larvae population

Fungi	Decrease of Larvae Population (%)					
	1 day	2 days	3 days	7 days	14 days	21 days
D. flagrans	58	45	30	9	0	0
V.chlamidosporium	62	56	38	11	4	0
F. solani	67	53	43	19	11	3
T. harzianum	69	58	50	25	17	8
Control	80	76	67	56	49	40

D. flagrans was able to kill 100% of larvae after 14 days of incubation. The number of the live nematodes larvae *was* decreased after challenging with *V. chlamidosporium, F. solani* and *T. harzianum* to 4, 11 and 17 respectively when incubated 14 days. *V. chlamidosporium* killed 100% of larvae after 21 days.

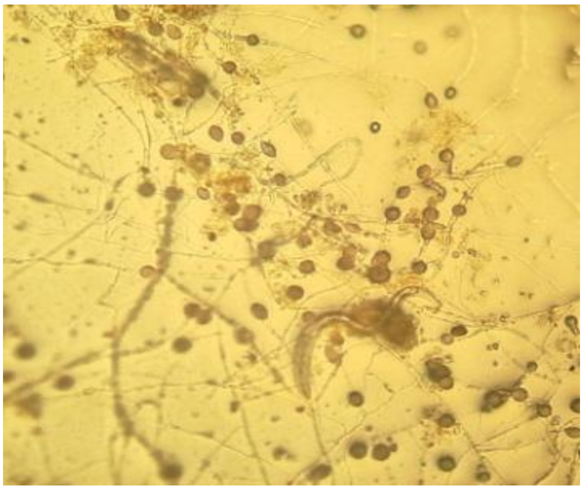

Figure 1: The trapped nematode larvae by D. flagrans

The significant effect was seen after 7 days of incubation, therefore, the live larvae was decreased to 9, 11, 19 and 25 for *D. flagrans, V. chlamidosporium, F. solani* and *T. harzianum respectively.* Figures 1 and 2 show the trapped larvae by 2 nematophagous fungi *D. flagrans* and *V. chlamidosporium*.

Figure 2: The trapped nematode larvae by V. chlamidosporium

Discussion

The most important purpose of biological control is to enhance the natural enemies of nematodes in environment subsequently as to decrease nematode density. Biological control of nematodes with fungi has been studied in several countries [12, 13].

The importance of the current work and similar studies is better understood when considered the free-living parasitic nematodes exist closely to our environment.

The *in vitro* efficacy of the genus *Monacrosporium* on Phyto nematodes, *Cooperia punctate* and *H. placei*, was demonstrated by Gomes et al. [14]. A study by Araújo et al. also confirmed the feasibility of using nematode-trapping fungus *Arthrobotrys robusta* in the biological control of parasite bovine gastrointestinal nematodes [15].

In our study, the use of *D. flagrans* for biological control of larva nematodes has great potential. The styles of traps have been investigated in detail in some predatory fungi. The adhesive network is the most extensive style used by nematode-trapping fungi.

Traps are the critical tools used by the nematode-trapping fungus to capture and kill nematodes [16]. Furthermore, traps are a significant marker for switching of nematode-trapping fungus from a saprophytic to a predacious lifestyle.

Our results illustrated that *D. flagrans* were most successful fungus for reducing the number of *Strongylidae* family larva stage from horse faeces. Follow *D. flagrans;* the live larvae was significantly reduced for *V. chlamidosporium, F. solani* and *T. harzianum* respectively.

However, *in vitro* studies on nematophagous property of fungi have limitations. They typically overrate the action of an agent by not allowing the larvae to escape. Lack of reproducing the interferences in soil and changes in the environment is another limitation of in vitro tests. Nevertheless, these methods have advantages, for example, a short assessment time and work between small physical spaces. Furthermore, the control of interaction between nematode and fungus is much easier. Further studies of the biological efficacy of nematophagus fungi in the field are required to obtain applicable strategy to control nematodes larvae contamination.

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