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Research Note

Inhibitory potential of selected biocontrol agents and plant pathogenic fungi against *Arthrobotrys musiformis:* An *in vitro* evaluation

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ABSTRACT: **An isolate of *Arthrobotrys musiformis* isolated previously was screened for its effectiveness against some potent plant pathogenic fungi and biocontrol agents. An *in vitro* assessment of the degree of inhibition suffered by *A. musiformis* in presence of fellow fungi indicated that this effective nematode predator could grow well in presence of all the fungi tested except *Trichoderma harzianum* isolates which inhibited the growth of *A. musiformis*.**

KEY WORDS: Antagonism, *Arthrobotrys musiformis*, biocontrol, integrated pest management

Parasitologists have identified several organisms that are effective natural enemies of nematodes (the main class being that of nematophagous fungi belonging to Deuteromycetes and Hyphomycetes) as classical biological control measures for Integrated Pest Management (IPM). Soil is a more complex environment biotically than abiotically and soil microflora compete with the introduced agent for food and significantly inhibits growth and activity of naturally occurring biocontrol agents. The basic knowledge of biology, ecology and potential of biological control agents is a preliminary requirement for their use as a successful component of IPM against plant and animal parasitic nematodes.

Haemonchus contortus – a round worm is a highly pathogenic, haematophagous gastro-intestinal parasite of small ruminants, specific to sheep and goats. *Arthrobotrys musiformis* was isolated from Gujarat and our previous laboratory studies indicated that it fulfills all the necessary criteria to be a potential biocontrol agent against *H. contortus* (Chauhan *et al.*, 2002a). However, for successful application under field conditions, it is also necessary to evaluate its performance in the soil ecosystem.

Among the plant biocontrol agents *Trichoderma* spp. and *Gliocladium* spp. are found to be potential biocontrol agents against a number of plant pathogenic fungi due to their prolific, rapid and uncontrolled growth in various natural soil followed by production of toxic organic metabolites, enzymes and antibiotics for

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mycoparasitism (Adriana & Sergio, 2000). Recently, *Paecilomyces lilacinus*, an egg parasitic fungi was also reported for its potential to be used as a component for the control of plant parasitic nematodes. *Arthrobotrys oligospora* and *Duddingtonia flagrans* have been studied more intensely for their exploitation as potential biocontrol agents against gastro-intestinal nematodes of ruminants (Sanyal, 2000).

In vitro studies on the performance of *Arthrobotrys musiformis* are essential for its effective use as a component of IPM in the soil ecosystem. Therefore, in the present study we evaluated the *in vitro* inhibitory potential of *A. musiformis* in the presence of commonly found plant pathogenic fungi (*Aspergillus niger, Lasiodiplodia theobromae, Fusarium oxysporum*) and plant biocontrol agents (*Trichoderma harzianum*: *Th-1*, *Th-2*, *Th-3* and *Paecilomyces lilacinus*) against these highly effective nematophagous fungi.

The inhibitory ability of the *Trichoderma harzianum* (*Th-1*, *Th-2*, *Th-3*), *Paecilomyces lilacinus*, *Aspergillus niger*, *Lasiodiplodia theobromae* and *Fusarium oxysporum* against *A. musiformis* was determined using 'Dual culture technique'. An agar disc of 8mm diameter containing pure cultures of each fungus was transferred to individual 2% corn meal agar (CMA) plates. After incubation at 25°C for one week, 8 mm discs from the peripheral growth of each fungi were transferred to fresh CMA plates in a paired combination with *A. musiformis* and antagonists, 25 mm apart from each other. Control plates of all the fungi were also maintained. Six replicates were set for each treatment. The data were collected after 5 days of incubation at 25°C and the means were based on 6 replicates for each treatment repeated thrice. Percent mycelial growth inhibition was determined by using the following formula:

$$M_i = [(M_a - M_b) \times 100] \div M_a$$

where,

M_i = Mycelial growth (mm^2) inhibition

M_a = Mycelial growth (mm^2) of *A. musiformis* in the absence of antagonists

M_b = Mycelial growth (mm^2) of *A. musiformis* in the presence of antagonists

As shown in the figure all the fungi tested exhibited different degree of inhibition of mycelial growth of *A. musiformis*. The result showed a strong suppression of mycelial growth of *A. musiformis* in the presence of all the isolates of *Trichoderma harzianum*, *viz.*, *Th-3*, *Th-1* & *Th-2* (98.89%, 98.77% and 98.57%, respectively), whereas *A. niger*, *F. oxysporum*, *L. theobromae* and *P. lilacinus* showed

Table 1. Growth profiles and per cent growth inhibition of *A. musiformis* in the absence and presence of fellow fungi

Sl. No.	Fungal Combination / Pairing	Radial growth in mm#	Growth inhibition of *A. musiformis* (%)
1	*A. musiformis* Control	31.6 ± 0.83	—
2	*A. musiformis* + *A. niger*	27.17 ± 0.80	26.07
3	*A. musiformis* + *F. oxysporum*	27.72 ± 0.72	23.05
4	*A. musiformis* + *L. theobromae*	29.94 ± 0.63	10.23
5	*A. musiformis* + *P. lilacinus*	26.5 ± 0.67	29.67
6	*A. musiformis* + *T. harzianum* – I	3.5 ± 0.31	98.77
7	*A. musiformis* + *T. harzianum* – II	3.78 ± 0.52	98.57
8	*A. musiformis* + *T. harzianum* – III	3.33 ± 0.44	98.89

Mean ± SD

relatively low suppression of mycelial growth of *A. musiformis* (26.07 %, 23.05 %, 10.23% and 29.67%, respectively) [Table 1].

Besides pH, temperature, heavy metals, soil environment greatly affects the establishment and activity of biocontrol agents added to soil. Therefore, it necessary to obtain basic information of their ecology and biology for its successful use as a component of IPM. Our previous study on *A. musiformis* indicated that it grows efficiently over a wide range of physico-chemical conditions such as pH, temperature, heavy metals (Chauhan *et al.*, 2002 a & b). But the present *in vitro* study on antagonism on *A. musiformis* indicated that it performs poorly in the presence of all the fungi tested.

Trichoderma, *Aspergillus*, and *Lasiodiplodia* are among the fast-growing fungi and can rapidly obscure colonies of other poorly growing fungi. Assessing the mechanism of action of each fungi was difficult as is the case of *T. harzianum* which antagonizes, inhibits and competes for scares of energy sources either as single or combining strategies (Adriana & Sergio, 2000).

Trichoderma spp. are currently being used in the soil as potential biocontrol agents against a number of plant pathogenic fungi. Their inhibition potential is also mounted against potential nematophagous fungi, *Arthrobotrys oligospora*. In the present study, inhibition of *Arthrobotrys musiformis* by *T. harzianum* clearly proves the nonspecificity of the latter.

The inhibition of mycelial growth of *A. musiformis* could be due to the production of toxic substances (enzymes, metabolites, antibiotics, volatile and non-volatile substances) by the antagonists that has been reported in *Trichoderma, Paecilomyces, Fusarium, Lasiodiplodia* and *Aspergillus spp.*

One of the most interesting observations in this study is that negligible growth inhibition of *A. musiformis* by *Lasiodiplodia, Aspergillus, Fusarium* and *Paecilomyces* though they produce toxins. This shows its competitive performance in such condition when they are in association.

The differences in inhibition of mycelial growth of *A. musiformis* in the presence of various fungal antagonists could be due to differential secretion of antifungal compounds. Rapidly growing fungi could also have caused nutrient depletion, which in turn leads to mycelial growth inhibition. This is the simplest mechanism used by some antagonists. Mukhopadhyaya *et al.* (2001) has also reported antagonistic effect of *Trichoderma harzianum* on growth of *Arthrobotrys oligospora* suggesting the strong antagonistic potential of former on the latter.

In general, it is considered that the performance of *A. musiformis* as a biocontrol agent against animal parasitic nematodes under soil environment depends on the absence of *Trichoderma* spp. which otherwise could alter the establishment and activity of nematophagous fungi. Further investigations are needed in order to observe/characterize the morphological changes (such as coiling, haustoria, disorganization of host cell content, penetration of the host) taking place during host-antagonist interaction observed during this study.

Trichoderma spp. have long been used as effective antagonists against several plant pathogenic fungi as it fulfills many criteria of potential biocontrol agent. The strong inhibition of *A. musiformis* by *T. harzianum* proves its nonspecificity. As *A. musiformis* competes poorly in the presence of *T. harzianum*, it is to be kept in consideration for its future use in the soil as a component of IPM against gastro-intestinal parasite of ruminants. Obviously studies related to the field testing of the nematophagous fungi must ensure absence of such antagonistic fungal genera that have the potential to adversely alter the performance of these otherwise beneficial fungi.

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