Biological Control 82 (2015) 76–85

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Assessment of germination and carnivorous activities of a nematode-trapping fungus *Arthrobotrys dactyloides* in fungistatic and fungicidal soil environment

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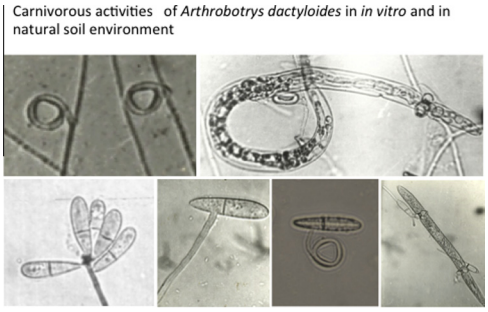
HIGHLIGHTS

- *Arthrobotrys dactyloides* was investigated for carnivorous activities in fungistatic and fungicidal soil.
- *A. dactyloides* was found a poor saprobe but an efficient nematode trapper in soils.
- Conidia bearing traps failed to grow in soil without capturing of nematodes.
- *A. dactyloides* found highly carnivorous to *Meloidogyne* spp. *in vitro*.
- Many fungicides found inhibitory to carnivorism of *A. dactyloides* in soil.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 June 2014
Accepted 19 December 2014
Available online 27 December 2014

Keywords:
Arthrobotrys dactyloides
Nematode-trapping fungus
Nematodes
Fungicides
Carnivorous
Root-knot nematodes

ABSTRACT

Carnivorism is the ability of nematode-trapping fungi to trap and digest the nematodes by sophisticated devices called traps. Delivery of nematode-trapping fungi in soil for bio-control of pest nematodes often fails or gives inconsistent results. Possible reasons for failure could be the effect of soil fungistasis on germination of nematode-trapping fungi in soil environment, use of avirulent species and sensitivity of these fungi to fungicidal residues in soil. Exploitation of nematode-trapping fungi for nematode control demands that it be compatible with fungicides applied in soil or crops and proliferate in soil. This investigation represents is one of the first to evaluate the effect of fungicides on the nematode-trapping fungus *Arthrobotrys dactyloides*. *A. dactyloides* showed *in vitro* carnivorous potential against *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne graminicola*, *Helicotylenchus dihystera* and *Heterodera cajani*. Conidia of *A. dactyloides* exposed to agricultural soils showed poor germination but formed conidial traps, which captured and killed the soil nematodes. Conidial traps, which trapped the nematodes, grew well in all soils after killing and nutrient absorption from nematode body. Soil amended with 20 mg ai kg^{-1} of carbendazim and thiram, 30 mg ai kg^{-1} of mancozeb, 50 mg ai kg^{-1} of captan, and 100 mg ai kg^{-1} of carboxin completely checked the conidial trap formation and nematode capturing. 30, 50 and 100 mg ai kg^{-1} of metalaxyl adversely affected the conidial trap formation and nematode capturing in soil. Propiconazole inhibited 15.2% conidial trap formation up to 50 mg ai kg^{-1} but caused 93.3% inhibition of conidial traps formation and complete inhibition of nematode capturing at 100 mg ai kg^{-1}. Sulphur, triademefon, and tricyclazole showed least toxic effect on conidial trap formation and nematode capturing activities of *A. dactyloides* in soil up to 100 mg ai kg^{-1}.

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http://dx.doi.org/10.1016/j.biocontrol.2014.12.014
1049-9644/

1. Introduction

Nematode-trapping fungi are a group of carnivorous fungi possess the unique feature of forming loop like infection structures called traps to capture, kill (paralyze) and digest the nematodes, rhizopodes and rotifers (Drechsler, 1937, 1941; Pramer, 1964; Thorn and Barron, 1984; Mcinnes, 2003; Barron, 1977). The interest of studying nematode-trapping fungi arises from their potential use as biological control agents of plant- and animal-parasitic nematodes (Kerry, 2000; Larsen, 2000). Nematode-trapping fungi occur in all sorts of soil environments where these fungi live both saprophytically and as facultative predators by capturing nematodes (Pramer, 1964; Nordbring-Hertz et al., 2006). To switch over to predacious lifestyle, these fungi forms traps on hyphae on or in which nematodes can be trapped either mechanically or by adhesion. These traps are adhesive or non-adhesive in nature and have thicker and more robust cell walls than typical vegetative hyphae (Higgins and Pramer, 1967). Nematode-trapping fungi produce sophisticated trapping structures including, one type of active mechanical trap known as constricting rings and five types of passive adhesive traps: sessile adhesive knobs, stalked knobs, adhesive nets, adhesive columns and non constricting rings. Non-constricting rings are always associated with the stalked adhesive knobs (Yang et al., 2012). Trap cells are different from the vegetative hyphae because of the presence of numerous cytosolic organelles called dense bodies and presence of a fibrillar layer of extracellular polymers, which are believed to be important for attachment of the trap cell to the nematode surface (Andersson et al., 2013). These traps are induced spontaneously, due to chemical compounds released from nematodes or some other factors (Dijksterhuis et al., 1994). After formation of traps, carnivorous activities precede a series of events including adhesion or trapping, penetration and immobilization of nematodes. At this stage the nematodes become paralyzed (killed), and their internal tissues are rapidly colonized by fungal hyphae (Dijksterhuis et al., 1994). Alternatively, traps may be formed directly upon conidial germination without an intermediate hyphal phase (Persmark and Nordbring-Hertz, 1997) or on short spore germlings (Cooke and Godfrey, 1964; Kumar, 2003) so-called conidial traps. These conidial traps are induced in response to cow dung (Dackman and Nordbring-Hertz, 1992), rhizosphere soil (Mankau, 1962; Persmark and Nordbring-Hertz, 1997) and are compound released by soil nematodes (Kumar, 2003). Formation of conidial traps by nematode-trapping fungi may give these fungi an opportunity to capture and extracts the abundant nutrition from the nematode body for growth and development in the soil having intense competition with other fungi for nutrients.

Plant-parasitic nematodes are known to cause global agricultural losses amounting to $157 billion annually (Abad et al., 2008). The root-knot nematodes (*Meloidogyne* spp.) *Heterodera cajani* and *Helicotylenchus dihystera* severely affect many economically important agricultural and horticultural crops worldwide (Wesemael et al., 2010; Zahid et al., 2002). Formulations of nematode-trapping fungi has been successfully applied in soil to improve plant health by suppressing root-knot nematodes (Stirling and Smith, 1998; Stirling et al., 1998; Kumar and Singh, 2006a; Singh et al., 2007, 2012), enhancing the accumulation of defense related biomolecules and induce systemic resistance in plants (Singh et al., 2013).

Arthrobotrys dactyloides Drechsler is a nematode-trapping fungus bears two celled straight or slightly curved conidia in open capitate arrangement (Drechsler, 1937). *A. dactyloides* also occasionally produces admixture of wider two-celled conidia with one large and one small cell as well as three-celled conidia with a larger middle cell (Kumar and Singh, 2006b). Conidial germination of *A. dactyloides* usually takes place with germ tubes developing into a hypha on which three celled constricting rings forms in presence of nematodes or in specific abiotic environments. When a nematode enters the constricting ring and contacts the inner surface of the ring cells, G protein-coupled receptors activate a down-stream signal pathway that includes cyclic adenosine monophosphate (cAMP), inositol-1, 4, 5-triphosphate (IP3), and Ca^{2+} (Chen et al., 2001). Subsequently, the ring cells rapidly (within 0.1 s) triple their volume and firmly constrict the nematode (Muller, 1958). *A. dactyloides* is also known to form conidial traps in response to soil (Dackman and Nordbring-Hertz, 1992) and soil nematodes (Kumar, 2003). *A. dactyloides* was found most effective against root-knot disease in tomato (Kumar and Singh, 2006a) and rice (Singh et al., 2007) in comparison to other nematode-trapping fungi.

Soil is a complex life supporting system in which a number of factors can influence the persistence or efficacy of bio-control fungi. For example, soil fungistasis which inhibits germination of most fungal propagules (Dobbs and Hinson, 1953). Unfortunately most of the bio-control fungi fail to germinate in soil due to soil fungistasis (Cooke and Godfrey, 1964; Mankau, 1962; Zhou and Mo, 2002; Bae and Knusen, 2002) and thus reduce the disease suppressive ability as expected. Similarly, fungicidal contamination of soil adversely affects the efficacy of bio-control fungi in soil (Crump and Kerry, 1986; Kidwai et al., 2006; Fravel et al., 2005). Introduction of nematode-trapping fungi to soil in the form of spores seems to be most feasible method for large-scale application of such organisms (Mankau, 1962). However, these fungi must have better adoptability in agricultural soils and be compatible with chemical pesticides that are applied in soil or crops to control other pest and diseases. Hence, the germination and carnivorous potential of nematode-trapping fungi in natural soils and fungicide contaminated soils need to be investigated to elucidate the fate of conidia applied in diverse situations of soil for bio-control purposes. Since, no work has been done so far on germination and carnivorous activities of *A. dactyloides* in fungicide contaminated soil and a little information is available on germination behavior and carnivorous activities of *A. dactyloides* in various type of agricultural soil, the present investigation was carried out to assess (i) temperature dependent radial growth and *in vitro* carnivorous potential of *A. dactyloides* against different species of plant-parasitic nematodes, (ii) germination and carnivorous activities of conidia of *A. dactyloides* in agricultural soils of different locations of India and (iii) germination and carnivorous activities of *A. dactyloides* in soil applied with different concentration of common fungicides.

2. Materials and methods

2.1. Fungal culture

A. dactyloides was isolated from the soil of citrus orchard of Narendra Deva University of Agriculture and Technology, Faizabad, India by the method described by Duddington (1955). Corn meal agar (CMA) medium (pH 7.0) was prepared with 20 g each of corn infusion and agar in one liter of distilled water and sterilized. 20 ml of CMA medium was poured into several Petri dishes. One gram of soil was sprinkled over the CMA medium and 1 ml water suspension having 2000–2500 saprophytic nematodes was inoculated in the Petri dishes as a prey for the nematode-trapping fungi. After occurrence of *A. dactyloides*, pure culture was made by harvesting spores from a conidial head by a fine needle and inoculating the same in Petri dishes containing corn meal agar medium. For identification of *A. dactyloides*, size of conidia, conidiophore, hyphae were measured and compared with the original descriptions given by Drechsler (1937) and Cooke and Godfrey (1964). Further, single spore culture was made by the method given by Singh et al. (2004).