

Study on Nutritional Requirements of Nematophagous Fungi in Terms of Carbon and Nitrogen Sources

Anamika¹

¹ Department of Agriculture, CAFF, Fiji National University, Fiji Island

Correspondence: Anamika, Department of Agriculture, CAFF, Fiji National University, Fiji Island. E-mail: anamika_bhu01@rediffmail.com

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Abstract

Nematophagous fungi act as natural enemies for biological control of plant parasitic nematodes. *Dactylaria eudermata* and *Arthropotrys oligospora* are the nematophagous fungi producing one to three dimensional hyphal baits and adhesive nets as a capturing device were selected and it was observed that growth and trapping effectiveness is influenced by temperature and C:N ratio. For the assessment of favorable temperature radial growth of both the fungi was studied at different temperature and 25 °C was recorded as an optimum temperature. Carbon and nitrogen content in media affect their predacity against plant parasitic nematode so their growth and sporulation were studied by providing different carbon and nitrogen sources. Among carbon sources, Glucose was the best source for both the fungi whereas Potassium nitrate for *D. eudermata* and Sodium nitrate for *A. oligospora* were recorded as the best Nitrogen sources.

Keywords: *Dactylaria eudermata*, *Arthropotrys oligospora*, temperature, carbon and nitrogen sources

1. Introduction

Nematode trapping fungi are responsible for keeping the nematode population in check and are an important part of the subsoil ecosystem. Nematophagous fungi predominantly present in the soil as saprophytes but act as parasites in presence of nematodes so as to fulfill the requirements of additional nutrient source in terms of nutritional advantage. The fungi converts its saprophytic stage to their parasitic phase and change their morphological structure into traps or mature spores. The development of infection structures is a prerequisite for the trapping of nematodes. The nematodes releases nemin like chemical substances which evokes the fungi to form trapping devices and these devices attracts nematodes towards itself and finally nematodes are trapped. If nematophagous fungi can be weaponized against nematode disease, then we will be able to see an even more noticeable difference. These fungi comprise more than 200 species found in all major taxonomic groups including lower (oomycetes, chytridiomycetes, zygomycetes) and higher fungi (ascomycetes, basidiomycetes and deuteromycetes). Most of them, including both nematode trapping and endoparasitic species, are asexual fungi (deuteromycetes), that all share the ability to attack living nematodes and use them as nutrients (Nordbring-Hertz et al., 2002). The nematophagous fungi, which infect nematodes with their conidia, are mostly more dependent on nematodes as a nutrient and are considered as obligate parasites (Jansson & Nordbring-Hertz, 1979; Jansson, 1982). These fungi are widely distributed in all types of agricultural soils but they are abundant in dung or surface litter and decaying plant debris. These fungi act on the principle of attraction, adhesion, penetration and killing live nematodes and digest it by producing fascinating trapping devices. The ability to capture nematodes is connected with the development of structures on the fungal mycelia. The nematophagous fungi have formed hyphal structures, such as adhesive hyphal nets, knobs, branches and constricting or non-constricting rings, in which nematodes are captured by adhesion or mechanically. Fungal hyphae then penetrate the nematode and utilize its content for food. Many nematode trapping deuteromycetes are indeed also good saprophytes and have evolved among cellulolytic or lignolytic fungi, they can use cellulose and other polysaccharides as carbon sources (Barron, 1992). The nitrogen content is less in plant debris and due to extremely low nitrogen levels, nematophagous fungi have evolved parasitic behavior to satisfy their nitrogen requirements as nutritional demands (Thorn & Barron, 1984; Barron, 2003). Also in the soil environments with a high carbon: nitrogen ratio, nematodes might serve as an important source of nitrogen. Part of the problem is that fungus population density, and especially trapping, is difficult to quantify in soil. Moreover, trapping usually

cannot be inferred simply from suppression of nematodes following organic amendment because such amendments can suppress nematodes in many different ways (Wang et al., 2002; Widmer et al., 2002). With organic matter of a C:N ratio greater than 20:1, N will temporarily be immobilized in microbial tissue, creating a nitrogen deficiency (Akhtar & Malik, 2000). The nematode management potential of an organic soil amendment is directly related to N-content or inversely related to the C:N ratio (Mian & Rodriguez-Kabana, 1982a, 1982b). Therefore it is necessary to study about the different sources of carbon and nitrogen with respect to the temperature as changes in climatic condition also affect the growth and development of the nematophagous fungi. So the objectives of the study were taken to determine the suitable temperature and appropriate carbon nitrogen sources for the radial growth of *D. eudermata* and *A. oligospora* *in vitro* condition.

2. Materials and Methods

Isolation of nematophagous fungi (*Dactylaria eudermata* and *Arthrobotrys oligospora*) was done from the soil sample collected from different villages of Allahabad, India by the method described by Duddington (1955). Single spore isolation technique (Tuite, 1969) was used for obtaining the pure culture of both *D. eudermata* and *A. oligospora* and were maintained on maize meal agar and used for further experiment.

2.1 Temperature Effect on Radial Growth of *D. eudermata* and *A. oligospora*

Maize meal agar medium was selected as an ideal medium to check the temperature effect on radial growth of both the fungi. 5 mm fungal disc of both the fungus were cut from the periphery of seven days old culture with the help of sterilized cork borer and inoculated into Petri dishes containing cooled and solidified maize meal agar medium and were incubated at 15, 20, 25, 30 and 35 °C.

Three petriplates were used for both fungi at varied temperature. Radial growths were measured on 6th days of inoculation and experiment was repeated three times in Complete Randomize Design and pooled data was subjected to statistical analysis.

2.2 Effect of Different Carbon Sources on Radial Growth of *D. eudermata* and *A. oligospora*

The radial growths of both the fungus (*D. eudermata* and *A. oligospora*) were studied on Yeast extract peptone soluble starch (Y PSS) medium. The carbon source of this medium soluble starch was replaced by five other carbon sources, viz. Dextrose, Sucrose, Glucose, Mannitol and Fructose. Medium with soluble starch served as check. All the culture media were prepared, sterilized and poured separately into several sterilized Petri dishes. 5 mm fungal disc, duly cut with a sterilized cork borer, were taken from the periphery of seven days old culture and inoculated into Petri dishes containing different media. Three replications were maintained for each treatment and were incubated at 25 ± 1 °C.

Radial growth and sporulation of both the fungus was measured after six days. The experiment was repeated three times in Complete Randomize Design and pooled data was subjected to statistical analysis.

2.3 Effect of Nitrogen Sources on Radial Growth of *D. eudermata* and *A. oligospora*

The radial growth of both the fungus (*D. eudermata* and *A. oligospora*) was studied on Czapek's medium. The nitrogen source of this medium sodium nitrate was replaced by five other nitrogen sources, viz. Calcium nitrate, Ammonium nitrate, Ammonium chloride, Potassium nitrate and Ammonium sulphate. Medium with Sodium nitrate served as check. All the culture media were prepared, sterilized and poured separately into several sterilized Petri dishes. 5 mm fungal disc were taken from the periphery of seven days old culture with a sterilized cork borer and inoculated into Petri dishes containing different media. Three replications for each treatment were maintained and incubated at 25 ± 1 °C.

Radial growth and sporulation of both the fungus was measured after six days. The experiment was repeated three times in Complete Randomize Design and pooled data was subjected to statistical analysis.

3. Results and Discussion

3.1 Temperature Effect on the Radial Growth of Nematophagous Fungi

The effect of temperature on the radial growth of the both nematophagous fungi are presented in Table 1 (Figure 1). Radial growth of the test fungi was significantly influenced by temperature. For the radial growth of the fungus at different temperature, 25 °C was recorded as an optimum temperature. From the observations recorded, it is quite clear that at this optimum temperature *A. oligospora* grows faster than *D. eudermata*. In general, radial growth of both the fungus showed decrease in growth with increase or decrease in temperature beyond the optimum temperature. At 30 °C the growth was appreciable while above 30 °C, the growth rate decreased significantly. At temperature below 20 °C, the growth of both the fungus was also very slow.

Table 1. Effect of temperature on growth of nematophagous fungi in MMA medium

| Temperature (°C) | Radial Growth (mm) After 6 Days | | Mean |
|---------------------|---------------------------------|----------------------|------|
| | Nematophagous fungi | | |
| | <i>D. eudermata</i> | <i>A. oligospora</i> | |
| T ₁ (15) | 15.2 | 16.2 | 15.7 |
| T ₂ (20) | 34.8 | 40.5 | 37.6 |
| T ₃ (25) | 69.1 | 78.2 | 73.6 |
| T ₄ (30) | 65.5 | 71.5 | 68.5 |
| T ₅ (35) | 58.2 | 60.2 | 59.2 |
| Mean | 48.5 | 53.3 | 50.9 |
| F – Test | S | S | |
| SEm± | 0.49 | 0.98 | |
| CD (P = 0.05) | 1.09 | 1.088 | |

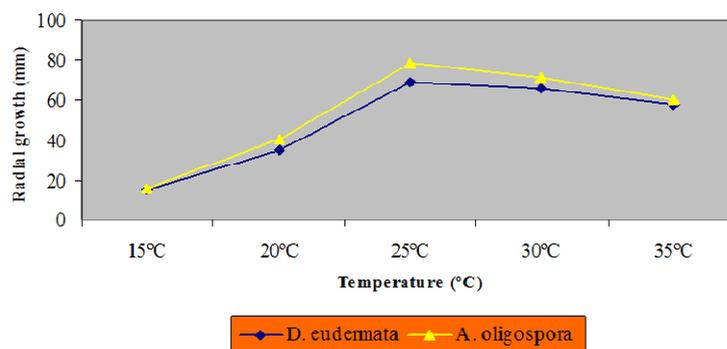


Figure 1. Effect of temperature on growth of nematophagous fungi in MMA medium

Similar observations on different temperature effect for different fungi have been reported by earlier workers. Pandey (1973) reported that 25 °C as optimum temperature for *Arthrobotrys robusta* while Gueye et al. (1997) found optimal growth of *A. musiformis* at 25-30 °C.

3.2 Effect of Carbon Sources on the Radial Growth of *D. eudermata* and *A. oligospora*

Data on the radial growth of *D. eudermata* and *A. oligospora* on different carbon sources are presented in Table 2 (Figure 2). Among the carbon sources, glucose supported maximum radial growth of *D. eudermata* followed by fructose. Mannitol, however, supported minimum growth in comparison to other carbon sources. Starch, sucrose and Dextrose also supported moderate growth of this fungus. The radial growth of *D. eudermata* on different carbon sources was recorded in the following order:

Glucose > Fructose > Starch > Sucrose > Dextrose > Mannitol

The test fungus *D. eudermata* sporulates well in all the test carbon sources. However, excellent sporulation of the fungus was observed on glucose followed by dextrose.

Glucose also supported maximum growth of *A. oligospora* followed by starch. Mannitol however, supported minimum rate of growth in comparison to glucose and Starch. Fructose, sucrose and dextrose showed appreciable growth of this fungus. The radial growth of *A. oligospora* on different carbon sources was recorded in the following order:

Glucose > Starch > Fructose > Sucrose > Dextrose > Mannitol

The test fungus *A. oligospora* sporulates in all the tested carbon sources. However, excellent sporulation of the fungus was observed in glucose and starch followed by fructose. Similar observation on glucose as best carbon sources for different fungi have been reported by earlier workers (Ajello, 1948; Colderone & Barnett, 1972;

Srivastava, 1981; Prasad, 1985; Singh, 2007).

Table 2. Effect of carbon sources on the radial growth of nematophagous fungi

| Treatments | Nematophagous fungi | | | |
|---------------------------|---------------------|-------------|----------------------|-------------|
| | <i>D. eudermata</i> | | <i>A. oligospora</i> | |
| | Radial Growth (mm) | Sporulation | Radial Growth (mm) | Sporulation |
| T ₀ (Starch) | 73.1 | +++ | 86.3 | +++++ |
| T ₁ (Fructose) | 75.1 | +++ | 83.2 | ++++ |
| T ₂ (Dextrose) | 65.9 | ++++ | 76.9 | ++ |
| T ₃ (Sucrose) | 70.1 | ++ | 81.2 | +++ |
| T ₄ (Mannitol) | 54.5 | + | 64.9 | + |
| T ₅ (Glucose) | 77.9 | +++++ | 89.1 | +++++ |
| Mean | 69.4 | | 80.2 | |
| F – Test | S | | S | |
| SEm± | 0.64 | | 0.68 | |
| CD (P = 0.05) | 1.39 | | 1.47 | |

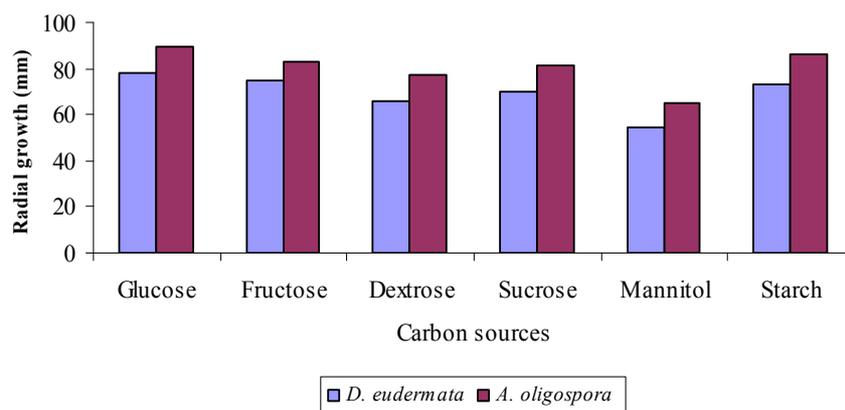


Figure 2. Effect of carbon source on the radial growth of nematophagous fungi

3.3 Effect of Nitrogen Sources on the Growth of *D. eudermata* and *A. oligospora*

Data recorded in Table 3 (Figure 3) shows that the radial growth of *D. eudermata* and *A. oligospora* differ significantly on different nitrogen sources. In case of *D. eudermata*, among six nitrogen sources, potassium nitrate supported maximum radial growth followed by sodium nitrate and calcium nitrate. Ammonium chloride was found to be the poorest source of nitrogen. The radial growth of *D. eudermata* on different nitrogen sources was recorded in the following order:

Potassium nitrate > Sodium nitrate > Calcium nitrate > Ammonium nitrate > Ammonium sulphate > Ammonium chloride

Among six nitrogen sources, sodium nitrate was found to be the best for the radial growth of *A. oligospora* followed by potassium nitrate and calcium nitrate while ammonium sulphate was found to be the poorest source of nitrogen. The radial growth of *A. oligospora* on different nitrogen sources were recorded in the following order:

Sodium nitrate > Potassium nitrate > Calcium nitrate > Ammonium nitrate > Ammonium chloride > Ammonium sulphate

Both the fungus sporulate in all the six nitrogen sources tested except *D. eudermata* does not sporulate on ammonium chloride, while *A. oligospora* sporulated comparatively lesser in ammonium chloride. Excellent

sporulation of both the fungus was observed in sodium nitrate and potassium nitrate. Similar observations on best nitrogen sources for different fungi have been reported by earlier workers (Lilly & Barnett, 1951; Srivastava, 1981; Prasad, 1985; Subramanian & Tyagi, 1968). The capacity of *D. eudermata* and *A. oligospora* to use nitrate indicate that the fungi produces reductase enzyme or other associated enzymes like hypernitrite reductase and hydroxylamine reductase, involved in the metabolism of the nitrate (Walker & Nicholas, 1962; Medina & Nicholas, 1957; Singh, 2007). The fungal growth in carbon and nitrogen deficient media may be accredited to the presence of these sources in form of impurities in other chemicals used during study.

Table 3. Effect of nitrogen sources on the radial growth of nematophagous fungi

| Treatments (Nitrogen Sources) | Nematophagous fungi | | | |
|------------------------------------|---------------------|-------------|----------------------|-------------|
| | <i>D. eudermata</i> | | <i>A. oligospora</i> | |
| | Radial Growth (mm) | Sporulation | Radial Growth (mm) | Sporulation |
| T ₀ (Sodium Nitrate) | 75.3 | +++++ | 88.5 | +++++ |
| T ₁ (Potassium Nitrate) | 78.6 | ++++ | 84.9 | +++++ |
| T ₂ (Calcium Nitrate) | 72.1 | +++ | 82.1 | +++ |
| T ₃ (Ammonium Nitrate) | 69.5 | ++ | 74.5 | ++ |
| T ₄ (Ammonium Chloride) | 61.2 | - | 68.2 | + |
| T ₅ (Ammonium Sulphate) | 64.5 | + | 64.5 | ++ |
| Mean | 70.2 | | 77.1 | |
| F – Test | S | | S | |
| SEm± | 0.44 | | 0.46 | |
| CD (P = 0.05) | 0.95 | | 1.00 | |

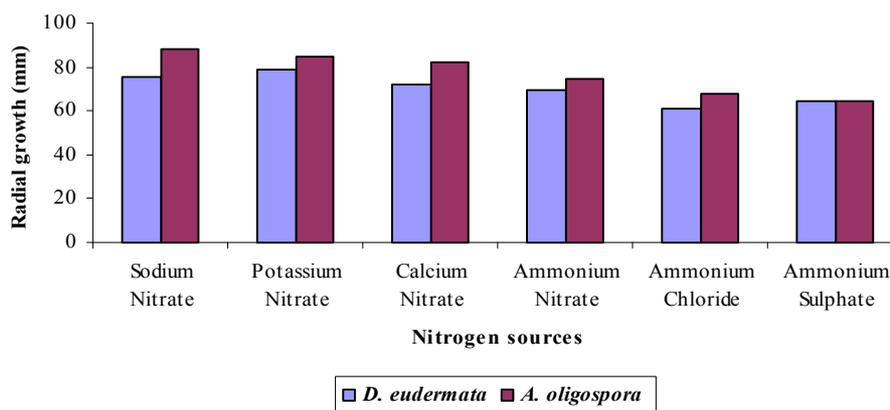


Figure 3. Effect of nitrogen sources on the radial growth of nematophagous fungi

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