ISOLATION, *IN VITRO* CHARACTERIZATION AND PREDACEOUS ACTIVITY OF AN INDIAN ISOLATE OF THE FUNGUS, *ARTHROBOTRYS OLIGOSPORA* ON THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

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Summary. An investigation was undertaken to isolate predaceous fungi from the soil and faecal matter of goat, sheep and cattle in the open grazing fields around Bangalore, Karnataka, India. An isolate of *Arthrobotrys oligospora*, PDBC AO1, was selected and the effects of temperature and pH on its development and its predaceous activity on *Meloidogyne incognita* were assessed under laboratory conditions. The fungus exhibited septate two-celled ovate-oblong conidiospores, trapping hyphae, and radiating and sparse mycelium. The optimum temperatures for its growth, sporulation, conidiospore germination and conidiospore production ranged between 25 and 35 °C, while pH optima ranged between 6.0 and 7.5. The optimum temperature for conidiospore germination was 25-30 °C. The isolate trapped 86% of second stage juveniles of the nematode and a maximum of 62% nematode juvenile mortality in 96 hours at 28 °C was recorded.

Among different biological control agents found active against several species of plant parasitic nematodes, fungi belonging to the class Hyphomycetes are not only ubiquitous in occurrence with wide host ranges and an amenability for mass production and commercial formulation, but are also effective against active stages of nematodes (Dreschler, 1937; Bordello *et al.,* 2002). Discovery, isolation and evaluation of such fungi and their geographical isolates (land races) for their effective use in regional and crop-specific nematode biocontrol programmes are expected to gain importance and relevance in organic farming. Therefore, the present study was carried out to ascertain its presence and to isolate the predaceous fungus, *Arthrobotrys oligospora* Fresinius, from grazing fields in and around Bangalore, India, and to study the effects of temperature and pH on its growth, sporulation, conidiospore production and predaceous activity.

MATERIALS AND METHODS

Isolation of the fungus. Forty soil and faecal (cattle, sheep and goat) samples were collected from the open grazing fields around Bangalore, Karnataka, India. Care was taken to collect soil along with the fresh droppings (faeces) of the milch animals. Samples were air dried for 6 hours at ambient room temperature (28-34 °C) and screened for the presence of predaceous fungi on 2% corn meal agar medium (CMA) (Himedia). Corn meal agar 2% (w/v) was autoclaved and streptomycin sulphate at 0.02% (w/v) was added to restrict bacterial growth. Air-dried faecal samples were gently pounded with a sterile pestle and mortar to obtain a powder of fine consistency. One gram of the powder from each sample was spread over the CMA medium (about 1 mm thick) in sterile Petri plates (9 cm-diam.) and incubated at 28 ºC for 15 days. Plates were then inoculated with approximately 250 freshly hatched second stage juveniles of *Meloidogyne incognita* (Kofoid *et* White) Citw. per plate (Duddington, 1955) and examined twice a week for three weeks under a stereomicroscope at 40× magnification. Four open Petri plates containing moist cotton swabs (6 cm diam.) were placed in the four corners of the incubator in order to maintain high relative humidity in the chamber. Predaceous fungi were detected by the presence of characteristic conidiospores and trapping structures formed around the trapped nematodes. Pure cultures were established by transferring these conidiospores to Petri plates containing sterile 2% CMA medium. Further identification was confirmed on the basis of the fungal morphology at 40-60× magnification (Dreschler, 1937; Haard, 1968). Only one isolate of *A. oligospora*, PDBC AO1, was obtained and this was used for further investigations.

Maintenance of *A. oligospora* for experimental use. The isolate of *A. oligospora* was grown on 2% CMA medium at 28 ºC for three weeks, until sporulation was completed. The conidiospores were then obtained by gently washing the culture plates with a small quantity of sterile distilled water containing 0.05% Tween 60 (Chandranathan *et al*., 1998). The numbers of conidiospores were counted using a haemocytometer.

Effect of temperature and pH on mycelial growth. Agar discs of 0.9 cm-diam. from the mother culture of the fungus were placed at the centre of 2% CMA plates

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(9 cm-diam.) and incubated at 15, 20, 25, 30, 35 or 40 ºC for seven days, with four replicates for each temperature. Similarly, the pH of another set of plates of CMA medium inoculated with the fungus was adjusted to 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 or 8.5 using 0.1N HCl and 0.1N NaOH. Fungal growth was assessed by measuring the diameter of the colony after seven days.

Effect of temperature and pH on the germination of conidiospores. The conidiospores obtained from the culture plates were used to make up a stock suspension. The conidiospore count was adjusted to about 100 conidiospores per 100 µl by serial dilution and checking the concentration using a haemocytometer. Aliquots of the stock suspension containing approximately 100 conidiospores were pipetted onto Petri plates containing sterile 2% CMA medium and these were incubated at 20, 25, 30 or 35 ºC for seven days.

Another set of Petri plates containing sterile 2% CMA medium at different pH (adjusted as described earlier) was inoculated with aliquots containing approximately 100 conidiospores and incubated at 28 ºC for seven days. The plates were periodically examined under a stereomicroscope to count the numbers of germinated conidiospores and individual colonies formed, and percentage germination was calculated.

Predaceous activity. The predaceous activity of *A. oligospora* was verified *in vitro* following a method similar to that described by Galper *et al.* (1995). Egg masses of *M. incognita* obtained from tomato roots were collected in sterile distilled water, placed on tissue paper and the eggs allowed to hatch at 28 ºC for 72 hours. The hatched second stage juveniles were collected and placed in 0.1% NaOCl for 45 seconds, washed 5 times in sterile distilled water and concentrated using a table-top centrifuge at 5000 rpm. The final juvenile suspension was diluted to give 1000 juveniles per ml. Aliquots of 100 µl containing 100 freshly hatched, healthy and surface-sterilized second-stage juveniles of *M. incognita* were added to Petri plates containing a fungal mat of *A. oligospora* on 2% CMA medium (approx. 1 mm thick and ten days old) and incubated at 28 ºC. The nematodes were examined under a stereomicroscope at 24-hour intervals for seven days to observe the trapping mechanism. The uncaptured juveniles were collected by gently washing the plates with sterile water. These uncaptured juveniles were counted and the percentage of captured juveniles estimated. The nematode suspension was also examined at 40× magnification to determine the number of nematodes washed from the plates that had fungal hyphae around their bodies and their number was added to the number of captured juveniles. Observations were recorded in four replicate plates and the mean values were estimated.

Data were subjected to analysis of variance and means separated according to modified Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The fungi isolated from the faecal matter were examined for mycelial and conidiospores characters under the microscope and several morphologically similar-looking fungi were screened. Only one isolate from the faecal matter of sheep matched the description of *A. oligospora* based on conidiospores, conidiophores and other morphological characters (Dreschler, 1937; Haard, 1968). Hyphae were sparse, characteristically radiated out from the point of origin or from the germinated conidiospores, and produced trapping rings or structures; conidiophores were elongated, slender, septate; conidiospores were septate, two-celled, ovate-oblong, and borne on sterigmata of conidiophores in loose clusters (Figs. 1 and 2). This isolate was designated as *A. oligospora* isolate PDBC AO1 and maintained at the Nematode Biocontrol Laboratory, PDBC, Bangalore.

Table I. Effect of temperature on mycelial growth, conidiospore germination, conidiospore production and sporulation of *Arthrobotrys oligospora* isolate PDBC AO1.

Temperature (ºC)	Conidiospore germination (%)	Mycelial radial growth (cm) (7 days of incubation)	Conidiospore production/ 9 cm-diam. Petri plate	Time to sporulation on CMA medium (days)
15	32 a	2.5 a	2.0×10^4 a	21 d
20	60 b	4.8 b	2.5×10^6 b	16 c
25	100 c	9.0 c	3.0×10^8 c	14 b
30	100 c	9.0 c	7.5×10^8 c	14 b
35	90 c	9.0 c	2.0×10^8 c	14 b
40	60 b	5.0 b	6.0×10^5 a	12 a
F-Test	*	*	*	NS
SEM ±	4.2	0.7	36.0	0.6
CD (P = 0.05%)	14.8	2.2	112.6	1.8

Values are means of four replicated plates.
* Significant at P = 0.05; NS = Not significant. Means followed by the same letter within a column are not significantly different according to modified Duncan's multiple range test.

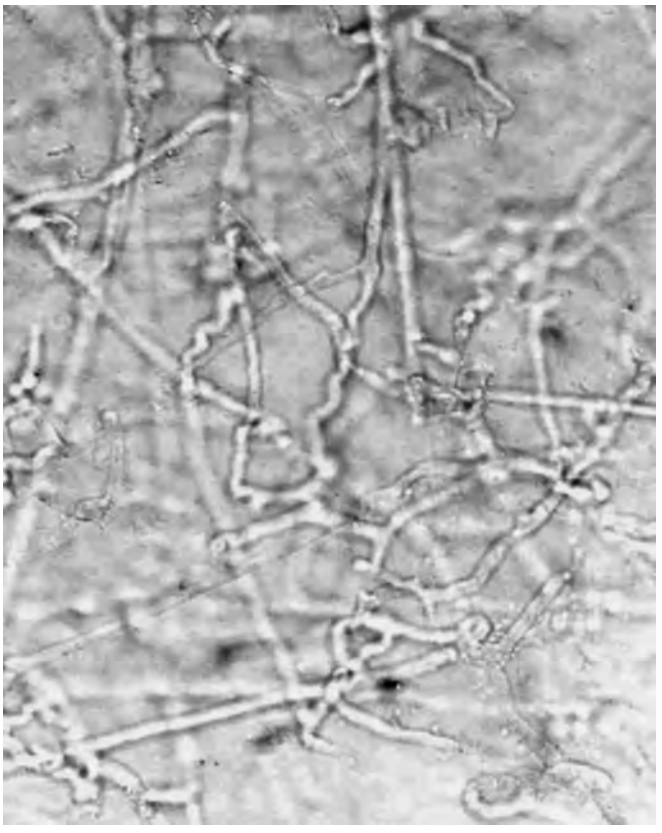

Fig. 1. Mycelium and hyphal trapping structures of *Arthrobotrys oligospora* isolate PDBC AO1 on 2% CMA medium (40×).

Fig. 2. Septate, two-celled oblong to ovate conidiospores of *A. oligospora* isolate PDBC AO1 on 2% CMA medium (40×).

The *A. oligospora* isolate PDBC AO1 exhibited germination of conidiospores, growth and sporulation at all temperatures tested (15-40 °C), with maximum values between 25 and 35 °C (Table I). 100% of the conidiospores germinated at 25 and 30 °C, with 90% germination at 35 °C. Similarly, mycelial growth on the CMA medium after ten days of incubation was maximum (9 cm) at 25, 30 and 35 °C, followed by 5 and 4.8 cm at 40 and 20 °C, respectively. Maximum conidiospore production, within the range 2.0 to 7.5×10^8 conidiospores per Petri plate was recorded at temperatures between 25 and 35 °C followed by 2.5×10^6 conidiospores per Petri plate at 20 °C. At 40 °C, the time taken for apparent completion of sporulation was only 12 days, while sporulation was completed in 14 days at 25, 30 and 35 °C. Gomez *et al.* (2003) reported that two Cuban isolates of *Arthrobotrys oligospora* showed greatest and least growth at 25 and 32 °C, respectively, and that conidiospore formation differed at varying temperatures. In the present study, *A. oligospora* isolate PDBC AO1 exhibited temperature optima for growth, conidiospore germination and conidiospore production between 25 and 35 °C on CMA medium. Castro *et al.* (2000) reported that, based on the regression analysis between fungal predation, migration and incubation temperature for fungal growth, the optimum temperature for *A. musiformis* predation on *M. javanica* was 20 °C. These studies clearly indicate that isolates of *A. oligospora* differ in their temperature optima, depending on their geographical origin.

Conidiospore germination, mycelial growth and conidiospore production were maximum at pH between 6.5 and 7.5, followed by that at pH 6.0 (Table II). Conidiospore germination was marginally less at acidic pH values of 5.5 and 6.0, but there was a significant reduction in germination at alkaline pH values of 8.0 and 8.5. Similarly, mycelial growth and conidiospore production at pH 8.0 and 8.5 were less ($P = 0.05$) than at acidic pH. Further, the time taken for apparent completion of sporulation was shorter, i.e. 15 days, at pH 6.5, 7.0 and 7.5 than at other pH values (22-23 days).

Time-course observations on the capturing (Fig. 3)

Fig. 3. Second-stage juvenile of root-knot nematode *Meloidogyne incognita* trapped by *A. oligospora* isolate PDBC AO1 (40×).

Table II. Effect of pH on mycelial growth, conidiospore germination, conidiospore production and sporulation of *A. oligospora* isolate PDBC AO1 incubated at 30 °C.

pH	Conidiospore germination (%)	Mycelial radial growth (cm) (7 days of incubation)	Conidiospore production/ 9 cm-diam. Petri plate	Time to sporulation on CMA medium (days)
5.5	90 b	7.5 b	3.0×10^6 b	23 b
6.0	90 b	9.0 bc	2.5×10^8 c	16 a
6.5	100 bc	9.0 bc	2.8×10^8 c	15 a
7.0	100 bc	9.0 bc	2.5×10^8 c	15 a
7.5	100 bc	9.0 bc	2.0×10^8 c	15 a
8.0	70 a	6.0 a	3.0×10^5 a	22 b
8.5	65 a	5.5 a	2.0×10^4 a	22 b
F-Test	*	*	*	NS
SEM ±	3.7	0.4	48.7	0.9
CD (P = 0.05%)	10.4	1.2	144.4	2.6

Values are means of four replicated plates.
* Significant at P = 0.05; NS = Not significant. Means followed by the same letter within a column are not significantly different according to modified Duncan's multiple range test.

and killing of *M. incognita* juveniles by *A. oligospora* isolate PDBC AO1 showed that the number of trapped nematodes increased with the time of exposure of the nematode to the fungus in the medium, from 22 to 86% over the period 48 to 96 h. This, in turn, was mirrored by an increase in the percentage of dead juveniles to 62% after incubation for 96 h (Fig. 4). Our results are similar to others reported previously. Galper *et al.* (1995) observed that fungi that formed hyphal networks took 24-48 h to trap more than 90% of *Caenorhabditis elegans* Maupas and *M. incognita* juveniles, and that fungi that formed constricting rings took 48-72 h to trap more than 90% of these nematodes. Rajeswari and Sivakumar (1999) reported that *A. oligospora,* a natural trap former, took 40 h to trap more than 90% of *Ditylenchus phyllobia* (Thorne) Filipjev.

In summary, the *A. oligospora* isolate PDBC AO1 exhibited optimum temperatures between 25 and 35 °C and pH optima between 6.0 and 7.5 for its growth, conidiospore germination, conidiospore production and apparent time taken for completion of sporulation. The isolate trapped 86% of *M. incognita* juveniles and recorded a maximum of 62% juvenile mortality in 96 hours at 28 °C on CMA medium. This information justifies the development of suitable mass production technologies, in order to evaluate the suitability of the fungal isolate for nematode biocontrol in specific agroclimatic situations.

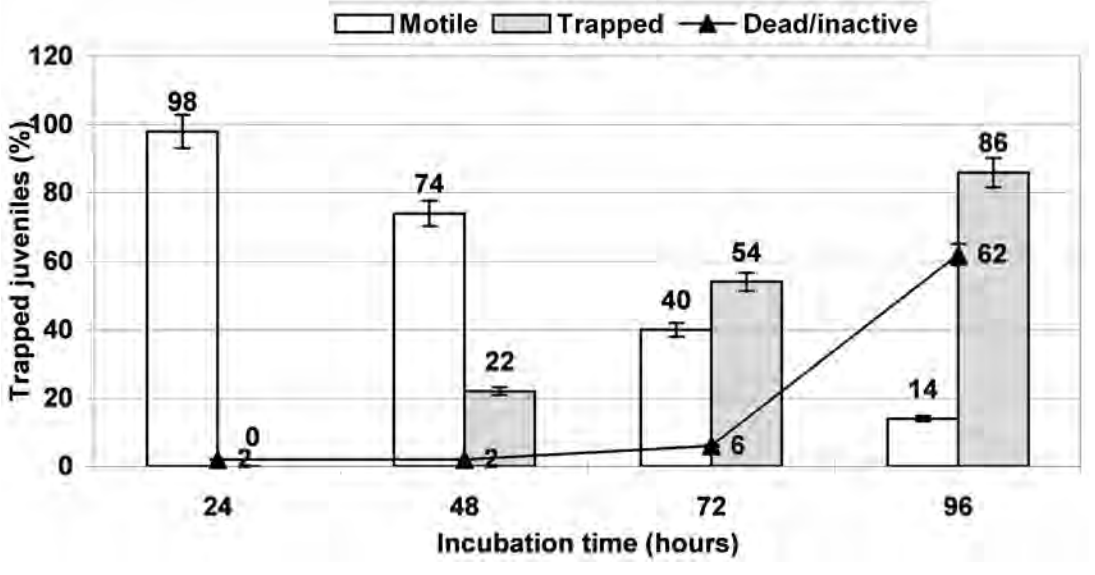

Fig. 4. *In vitro* bioefficacy of *A. oligospora* isolate PDBC AO1 against *M. incognita* juveniles on CMA medium at 28 °C.

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