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Biological Characteristics and Effects of Two Strains of *Arthrobotrys oligospora* from Senegal on *Meloidogyne* Species Parasitizing Tomato Plants

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Two strains (ORS 18692 S7 and ORS 18692 S5) of the nematophagous fungus Arthrobotrys oligospora *have been isolated for the first time in Senegal.* In vitro, *both strains trapped 98% of groups of 7-day-old* Meloidogyne mayaguensis *juveniles within 48 h. Their optimal growth occurred at 25–30°C and at a pH of 5.6, but salinity inhibited their development. In order to test the fungi on* M. mayaguensis *in pots and in mixed populations of* Meloidogyne *spp. on tomato in the field, the fungus was incorporated into compost blocks before sowing and subsequent transplanting. In pot experiments during the cool season (≤ 28°C), both strains reduced nematode populations and stimulated seedling growth. In field trials during the hot season (≤35°C), both strains were efficient in reducing* Meloidogyne *populations. The feasibility of combining the use of compost blocks with the introduction of* A. oligospora *is discussed.*

Pour la première fois, deux souches du champignon nématophage Arthrobotrys oligospora *(souches ORS 18692 S5 et ORS 18692 S7) ont été isolées au Sénégal dans des zones de culture maraîchère.* In vitro, *l'activité prédatrice des 2 souches envers* M. mayaguensis *s'est révélée très importante après seulement 48 h de confrontation. La croissance de ces souches est maximale entre 25 et 30°C, à pH 5.6 mais elle est inhibée par la salinité. Afin d'étudier, sur tomate, leur capacité à contrôler une population de* M. mayaguensis *en pots et de populations mélangées de* M. incognita, M. javanica *et* M. mayaguensis *au champ, les souches ont été incorporées à des mottes de compost avant semi. En pots, et pendant la saison fraiche (≤ 28°C), toutes les souches ont inhibé le développement des nématodes et ont permi une stimulation de la croissance des plants. Au champ, et pendant la saison chaude (≤ 35°C), les souches ORS 18692 S5 and ORS 18692 S7 se sont révélées très efficaces. La mise en œuvre d'une technique de pépinière combinant l'utilisation des plants en motte et l'incorporation de souches indigènes d'*A. oligospora *est discutée.*

Keywords: Arthrobotrys oligospora, *biocontrol, compost,* Meloidogyne mayaguensis, Meloidogyne *spp., Senegal; tomato*

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INTRODUCTION

Plant parasitic nematodes, especially root-knot nematodes (*Meloidogyne* spp.), are cosmopolitan and important problem pests affecting the production of both subtropical and tropical crops (Johnson & Fassuliotis, 1984). To a large extent, nematode control methods have been based on the use of chemicals applied to the soil or the plant. However, they are expensive and very toxic when improperly used. Public concern over the possible damage caused by toxic pesticides and the continued intensification of crop production has promoted research into biological control methods. The use of microorganisms to control plant parasitic nematodes has, among others, focused on the application of nematode-trapping fungi. *Arthrobotrys* spp. (hyphomycetes) have been tested against *Meloidogyne* spp. (Mankau, 1961; Vouyoukalou, 1993). *A. irregularis* has provided significant control of *M. incognita* on tomato (Cayrol, 1983; Pelagatti *et al.*, 1986). In Senegal and in sahelian regions in general, members of the genus *Meloidogyne* are the most widespread nematode pests in vegetable-producing areas. Nevertheless, the nematode-trapping fungi have not been studied, and their potential for nematode control is unknown in these countries.

In order to develop biological control methods for nematodes, a survey of trapping fungi *Arthrobotrys* spp. was undertaken in Senegal. A study was initiated to investigate:

(1) the effects of temperature, pH and salinity on the *in vitro* growth of strains of the fungi;
(2) the potential for trapping *M. mayaguensis*, commonly found on vegetables in Senegal; and
(3) the development of a control method using *A. oligospora* incorporated into compost blocks to control *M. mayaguensis* populations in both pots and mixed populations of *M. incognita, M. javanica* and *M. mayaguensis* in the field.

MATERIALS AND METHODS

Isolation, Culture and Characterization of *Arthrobotrys* sp. Strains

Fifty soil samples from several vegetable-producing areas in Senegal were analyzed. Petri dishes were filled with diluted brewery wort (1.8–2.0 g l^{-1} total sugars after dilution, pH 5.5) and solidified with 20 g l^{-1} of agar. The plates were sprinkled with 1 g of soil using the technique of Drechsler (1941). After 7 days at 25°C, the fungi were well developed and produced erect conidiophores. Single conidia were picked off under the dissecting microscope and transferred to Petri plates filled with diluted brewery wort. These single-spore cultures were maintained aseptically in the dark at 25°C.

Interest was focused on *Arthrobotrys* spp. because of this organism's practical potential for the control of root-knot nematodes, which has been demonstrated in high-value crops (Cayrol & Frankowski, 1980). Five isolates of *Arthrobotrys* spp. were obtained from the 50 soil samples. Two of them (ORS 18692 S5 and ORS 18692 S7) were selected for their fast growth, and were used in the experiments. These two fungal isolates have been characterized as *A. oligospora* Fresenius by the Centraalbureau voor Schimmelcultures (the Netherlands). The fungi were cultured using the technique described above, but were inoculated on to the surface of a sterile piece of filter paper which was placed on the culture medium. Mycelium of each isolate was taken from the surface of the paper and extracted in a Tris-HCl buffer (pH 8) containing 17% sucrose, 1.8% ascorbic acid and 1.4% cysteine-HCl (Trudgill & Carpenter, 1971). After centrifugation for 15 min (10 000 × *g*), the supernatant was analyzed by electrophoresis using the technique developed for nematodes by Dalmasso and Bergé (1978) in polyacrylamide gel (7%, pH 8.4). β-esterases were revealed using 1-naphtyl acetate and Fast-Blue R as the staining reagent (Brewer & Singh, 1970).

Study of Factors Influencing Growth *in Vitro*

Temperature. Agar plugs were taken from the margin of the fungal colonies and transferred to Petri dishes (90 mm in diameter), filled with brewery wort (adjusted with sodium phosphate buffer, pH 7, and for 1.8–2.8 g l^{-1} of total sugars) solidified with 20 g l^{-1} of agar. Petri dishes were incubated at 25, 30, 35 and 40°C for 1 week in the dark. Each treatment was replicated five times. During the week, daily measurements of the diameter of each colony were made using the mean of two measurements at 90°, and their overall mean was calculated. The data were analyzed by a one-way analysis of variance and the mean values were compared with the Student's *t*-test ($P < 0.05$).

Salinity. Plugs from the same cultures were transferred into Petri dishes filled with the medium described (pH 7) and supplemented with four concentrations of sodium chloride (NaCl) (0.00, 0.58, 1.46 and 2.92 g l^{-1}). Each treatment was replicated five times. The effects on the fungal growth were measured and calculated as above after 5 days in the dark at 25°C.

pH. Plugs of the fungal cultures were transferred to Petri dishes filled with the medium described above, adjusted to pH 5.6, 6.8 or 7.8 using different sodium phosphate buffers (Dawson *et al.*, 1969). The cultures were incubated at 25°C for 1 week in the dark. Each treatment was replicated five times. During the week, the effect of pH on fungal growth was measured daily as above.

***In Vitro* Tests for Predacious Activity against Nematodes**

A population of *M. mayaguensis* was reared on tomato (*Lycopersicon esculentum* Mill.), cv. Roma. Two months after inoculation, the roots were sampled, cut into short pieces and placed in a mist chamber for 1 week to enable the nematode eggs to hatch.

Each fungus was cultured on the medium described above (pH 5.6, 25°C) for 2–3 weeks. Agar plugs (6 mm in diameter) were taken from the margin of the fungal colonies and transferred to Petri dishes (90 mm in diameter) filled with distilled water agar (20 g l^{-1}).

One week later, 100 7-day-old second stage juveniles were introduced in a water droplet on to the fungal cultures. Treatments were replicated five times. After 24 and 48 h, the juveniles trapped by the fungus were counted using a dissecting microscope. The trapping rates (trapped juveniles/total juveniles) were transformed by arcsin($\sqrt{}$ rate) and analyzed by a one-way analysis of variance. The mean values were compared with the Student's *t*-test ($P < 0.05$).

Pot Experiment

Solid fungus inocula were prepared in 0.5-dm^3 glass flasks containing 0.3 dm^3 of compost (Table 1) made from a mixture of cattle and sheep offal (SERAS company, Thies, Senegal). This subtrate was moistened with liquid brewery wort (18–20 g l^{-1} of total sugars) and inoculated with plugs from an agar culture of each *A. oligospora* isolate. The cultures were incubated for 5 weeks at 25°C. Afterwards, they were diluted with the same compost free of fungus (1:10, v:v). This mixture was used to make small blocks ($4 \times 4 \times 4$ cm^3) with a mechanical apparatus (FAO patent, made by SAMA, Dakar, Senegal). Tomato seeds were sown in these blocks, and seedlings were grown for 3 weeks. Then they were transferred to 0.5-dm^3 pots filled with autoclaved soil (Table 1). Control treatments were seedlings directly transplanted without compost blocks, or transplanted with blocks free of fungus. The seedlings were maintained outside from January to February (maximal temperature ≤ 28°C) and watered twice a week. They were inoculated with 5-ml suspensions of 100 7-day-old second stage juveniles of *M. mayaguensis* or water alone 3 weeks after sowing. Nematode suspensions were poured into the soil around the compost blocks. The pots were arranged in a randomized, complete block design with seven replicates.

Two months after nematode inoculation, the seedlings were uprooted and their roots were washed. Shoots were dried at 65°C for 1 week and weighed. Galls induced by *M. mayaguensis* were rated using Zeck's scale (1971). The roots were then cut into 1–2-cm pieces and placed in a mist chamber for 2 weeks to recover hatched juveniles (Seinhorst, 1950). The roots were oven

TABLE 1. Characteristics of the site from which both strains of *A. oligospora* were isolated and physico-chemical characteristics of the subtrates used in experiments

Characteristics[a]	Compost	Soil	Soil of fungal strains
Texture (%)			
Clay (0–2 μm)		3.9	4.3
Silts (2–50 μm)		2.9	2.7
Sands (50–2000 μm)		92.2	93.0
Organic matter			
Carbon (%)	369	3.73	5.7
Nitrogen (%)	20	0.45	0.72
C/N	18.45	8.3	7.9
Minerals			
NaCl (mg dm^{-3})	—	—	304
Total cations (meq%)	3.6	10.0	8.33
Cation exchange (meq%)	0.9	4.66	2.37
Total P_2O_5 (ppm)	24000	352	518
pH	7.5	7.75	6.30

[a]Concentration (mg dm^{-3}) corresponds to the conductivity tested (mS).

dried and weighed. A subsample of soil (250 g) was taken from each pot and the nematodes were extracted using an elutriator (Seinhorst, 1962).

The data were treated with a one-way analysis of variance and the mean values were compared with the Student's *t*-test ($P < 0.05$). For nematodes, data were previously transformed by log $(x + 1)$.

Field Experiment

Tomato seedlings were grown for 3 weeks in compost blocks with or without fungi, prepared as described above. The seedlings were then transferred to plots (4 × 5 m; 45 plants/plot, 0.5 m apart) separated from one another by 2-m paths. The treatments, arranged in a randomized, complete block design with six replicates, consisted of the two fungal strains and a control (no fungus). This trial was conducted from August to September (maximal temperature ≤35°C) on a sandy soil (2.7% clay, 1.5% silt, 95.8% sand, and 4.5% organic matter, pH 7.06). In a previous crop, it had been observed that this field was highly infested with nematodes (51.6% *M. javanica*, 41.3% *M. mayaguensis* and 7.1% *M. incognita*). No nematophagous fungi have been detected in this field. Every 10 days from transplanting to the end of the harvest, the soil and roots from two plants/plot were mixed, and the nematodes were extracted as described above. When 50% of the remaining plants were flowering (flowering date), root galls were indexed according to Zeck (1971). The total number of nematodes dm^{-3} of soil and nematodes g^{-1} of roots (cumulative nematode population from planting to harvest) were treated with a one-way analysis of variance, and the mean values were compared using the Student's *t*-test ($P < 0.05$). Data were previously transformed by log $(x + 1)$.

RESULTS

Characterization of the Fungal Strains

Two different esterase phenotypes were obtained (Figure 1) which could be used to distinguish the two isolates by the number and the rate of migration (*Rm*) of the bands of the isozymes. The two strains were characterized by the following patterns: four bands for ORS 18692 S5 (0.38, 0.74, 0.77 and 0.81) and three bands for ORS 18692 S7 (0.66, 0.75 and 0.85). These two isolates were different, but they were isolated from the same soil sample (Table 1).

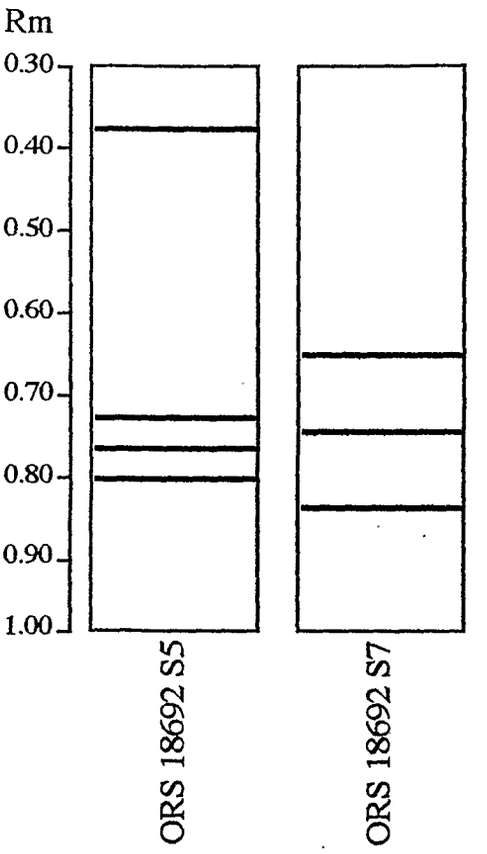

FIGURE 1. Esterase phenotypes of both isolates of *A. oligospora* (*Rm* = relative migration).

Effects of Temperature, pH and Salinity on Fungus Growth

The fungal radial growth was significantly greater at 25 and 30°C than at the other temperatures (Table 2). At 35 and 40°C, the two strains did not grow. Both isolates grew better on an acidic medium (pH 5.6) than on a basic one (pH 7.8). The fungal growth always decreased when salinity increased. There was no significant difference in growth at concentrations of 0.58 and 1.46 g l^{-1} of NaCl.

Trapping Activity of the Fungi

At 25°C, both fungal isolates trapped the majority (87%) of *M. mayaguensis* juveniles within 24 h (Table 3). After 2 days, 97 and 98% of the nematodes had been trapped by ORS 18692 S5 and ORS 18692 S7 respectively (Table 3).

Plant Growth and Nematode Development in Pots

The shoot weights of the plants receiving no inoculum were significantly greater than those which were inoculated with *M. mayaguensis* (Table 4). Plants grown in compost blocks which were transferred to soil inoculated with nematodes had a gall index and multiplication rate which were significantly smaller than those on plants growing without compost blocks.

The shoot biomass was not different in treatments where plants were grown in soil or compost blocks, but root biomass was significantly greater when plants were grown in compost blocks. When the plants grown in compost blocks (inoculated with the fungal isolates and transferred to soil) were inoculated with nematodes, the gall indices and the multiplication rates were significantly lower than those measured on plants without fungi. There was no difference between the effect of the two isolates. With both strains, the shoot biomasses were greater than

TABLE 2. *In vitro* influence of temperature, pH and salinity on the radial growth (mm) of the *A. oligospora* strains used in the study

	Growth of *A. oligospora* strains (mm)[a]	
Culture conditions	ORS 18692 S5	ORS 18692 S7
Temperature (°C)		
25	86.6a	85.9a
30	89.3a	8.0a
35	NG[b]	NG
40	NG	NG
pH		
5.6	70.8a	79.4a
6.8	66.0b	75.8a
7.8	48.8c	59.5b
Salinity (g dm^{-3})		
0.00	68.9a	63.4a
0.58	35.8b	29.6b
1.46	38.8b	32.5b
2.92	20.9c	14.4c

[a]For each condition and fungal isolate, data followed by the same letter are not significantly different ($P < 0.05$).
[b]NG = no growth.

those of plants grown in compost blocks without fungi, and were the same as those of plants free of nematodes. The root weights were greater only when protected with the strain ORS 18692 S7.

Nematode Development in the Field

In the roots, the nematode infestation of the control plants (no fungi) increased 10 days after planting until the 50% flowering stage, and then did not increase until and after harvest. In the soil, no juveniles were detectable for 20 days after planting (Figure 2). Then, the infestation increased for 20 days until harvest. The fluctuations of the nematode populations were the same in roots and soil, but with a delay of 10 days in the soil. The mean gall index of 4 occurred at 50% flowering (Figure 2).

Nematode populations in the presence of fungi fluctuated in the same way. Comparing the effects of the strain ORS 18692 S5 with the control (Figure 2), the fluctuation of the nematode populations was the same in the roots and in the soil; the nematode populations increased more

TABLE 4. Effects of the *A. oligospora* isolates on the development of *M. mayaguensis* populations and on the growth of infested tomato plants in pots 2 months after inoculation[a]

A. oligospora strain	Substrate	Inoculum	Gall index	Multiplication rate[b]	Biomass (g)	
					Shoots	Roots
No fungus	Soil	0	0a	0a	2.3b	1.11b
No fungus	Soil	100	4d	88.5d	1.42a	0.62a
No fungus	Compost + soil	100	3c	26.7c	1.72a	1.35c
ORS 18692 S5	Compost + soil	100	2b	7.8b	2.49b	1.50cd
ORS 18692 S7	Compost + soil	100	2b	9.4b	2.83b	1.65d

[a]For each treatment combination, data followed by the same letter are not significantly different ($P > 0.05$).
[b]Multiplication rate = (total number of juveniles in soil and roots)/inoculum.

on plants inoculated with ORS 18692 S5 than on plants free of fungi. The development of the populations was less on the plants inoculated with ORS 18692 S7 in soil and roots than than on the plants of the control treatment.

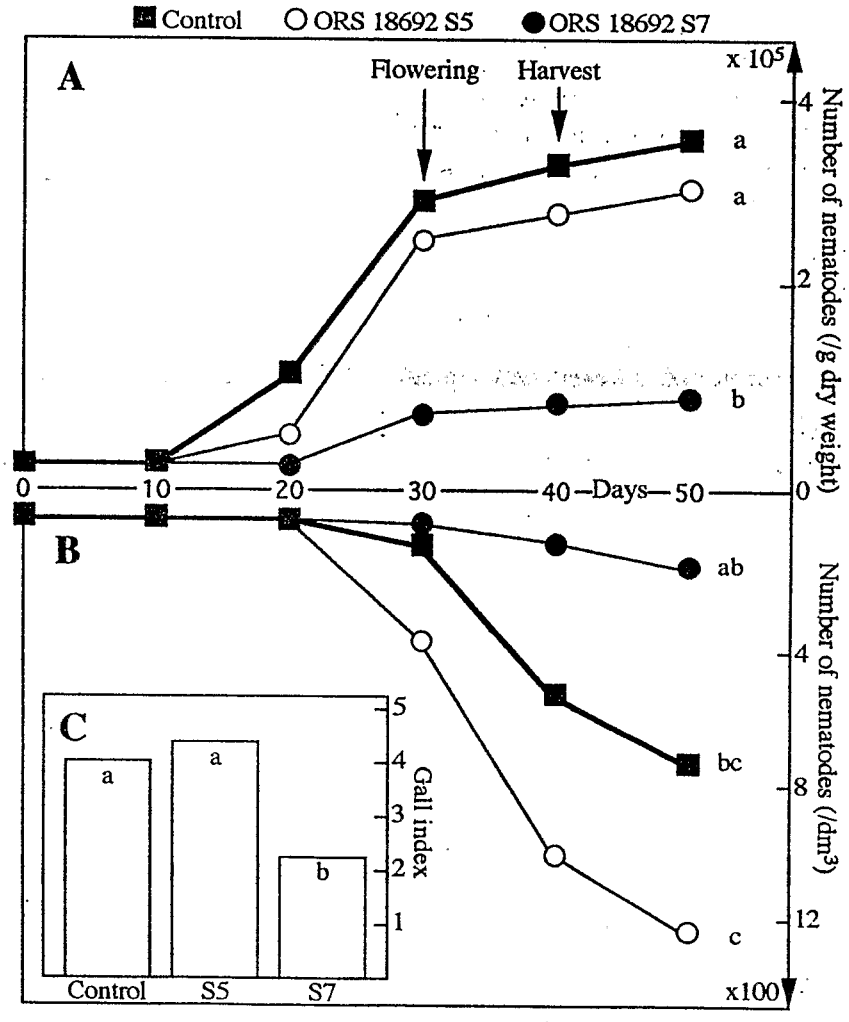

FIGURE 2. Changes of populations of *Meloidogyne* spp. juveniles in tomato roots (a) and in the soil (b), and the effects of both isolates of *A. oligospora* on the gall index (c) at flowering in the field. Data with the same letters are not significantly different ($P < 0.05$).

DISCUSSION

The isolates of *A. oligospora* found in Senegal can control the multiplication of *M. mayaguensis* parasitizing vegetables. This very damaging *Meloidogyne* species (Rammah & Hirschmann, 1988) was chosen because it has spread throughout the most important vegetable-producing areas in Senegal (Mateille *et al.*, 1994), and is now present under various agro-climatic conditions.

In vitro trapping tests prove that these fungal isolates are very efficient, but *in situ* trials show that their efficacy depends very heavily on environmental factors. However, no tests have been performed yet to verify their effect on *M. incognita* and *M. javanica.*

The growth of both isolates is depressed by the presence of sodium salts in the culture medium. Indeed, these fungi have been isolated in soils with low salinity levels. In Senegal, therefore, it could be difficult to introduce such fungi into the vegetable producing areas with high salinities. These areas are just by the sea in the Niayes region where the salinity has increased in recent years due to the extraction of ground water for irrigation. The problem is emphasized by the fact that *Meloidogyne* species, which are usually known to be sensitive to high salt concentrations (which create sufficient osmotic pressure to inhibit hatching (Prot, 1978)), seem to be well adapted to the local conditions. Their root populations are consequently very large.

Generally, nematodes aggregate in a pH range from 5.8 to 8 when they are placed in a pH gradient (Jairajpuri & Azmi, 1978). In Senegal, almost all vegetables are grown in acidic soils. Since fungal isolates of *A. oligospora* grow best on acidic (pH 5.6) media, they are well adapted to these soil conditions. In the pot experiment, the pH of the substrate of the soil (7.75) and compost (7.5) was approximately neutral, and was not well suited. Nevertheless, the fungal isolates reduced nematode infestations and stimulated plant growth.

Compared with salinity and pH, the influence of temperature seems to be more important for the growth of the fungi and their activity against nematodes. The *in vitro* temperature threshold is very narrow, between 25 and 30°C for most of the isolates, and the mean lethal temperature of 35°C is, in contrast, the optimal temperature for most of the *Meloidogyne* species in the tropics (Netscher & Sikora, 1990). During the pot experiment, the temperature did not exceed 28°C. The fungi were therefore in the best conditions for growth, and were all efficient in reducing *M. mayaguensis* populations and allowed better plant growth. In the field trial, the temperatures exceeded 30°C, but this cannot explain the differences between the efficiency of both strains.

The success of the establishment of nematophagous fungi in soils depends on environmental factors and on the mode of introduction, especially the incorporation of suitable nutrients. In this study, the choice of a compost made from a mixture of cattle and sheep offal was very successful and well suited to these fungi, which colonized all the substrate (0.3 dm^3) within 1 month. It is well known that organic matter acts as an energy source for nematophagous fungi. However, such additions can induce direct effects on nematode populations (Akhtar & Alam, 1993) through the production of secondary breakdown products. This could have been the case during the pot experiment, in which the addition of organic matter alone decreased the development of *M. mayaguensis* populations. The experiment revealed that the combined effect of organic matter and fungus was greater than that of organic matter alone. From a practical point of view, this technique provides four advantages. Compost blocks provide better seedling growth in the nursery, they provide useful organic amendments, induce a significant protection against nematodes and could provide a good and simple means of dissemination for nematophagous fungi in tropical vegetable-producing systems. The development of the fungi on roots beyond the compost block has not been studied. However, after 1 week of culture, the roots were beyond the compost block, and, presumably, the fungus has colonized and protected new roots for the duration of the experiment. These fungi can control *M. mayaguensis* populations. As this nematode can parasitize some plant species or cultivars commonly resistant to other tropical *Meloidogyne* species (e.g. *M. incognita* or *M. javanica*), it is more difficult to control with crop rotation. Biocontrol with nematophagous fungi is a promising technique which may be introduced in integrated pest management for root-knot nematodes. Therefore, more experiments

should be performed in order to verify the effects of these fungal strains during the cool season (between November and March) and on different types of soil, to determine the inoculum levels of the fungi. Also, there is a need to explore the possibility of producing the most effective strains through simple technologies adapted to local industrial conditions, with the cultural practice of the compost blocks. This study shows that the use of indigenous strains of *A. oligospora* is of interest in developing countries.

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