

RESEARCH / INVESTIGACIÓN

Assessing the predatory activity of *Arthrobotrys oligosporus* strain C-2197 as biocontrol of the root-knot nematode *Meloidogyne* spp.

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Abstract: The root-knot nematode, *Meloidogyne* spp., is an endoparasite that infects plants' root system and causes yield losses in several important crops. *Meloidogyne* is one of the most devastating pests, so searching for effective biological agents is needed to mitigate its damage. In this study, the predatory activity of *Arthrobotrys oligosporus* Fresen strain C-2197, obtained from a tropical dry forest of Ecuador, was evaluated as a biocontrol alternative for root-knot caused by *Meloidogyne* spp. Our results showed that *A. oligosporus* C-2197 has predatory activity against juvenile nematodes, 72.31%, and 79% efficacy, for *in vitro* and greenhouse conditions. Besides, the studied strain showed growth-promoting activity, increasing leaf and root area of inoculated plants. Growth promoting activity was also observed in field tests. The present study validates the potential use of *A. oligosporus* as a biocontrol of *Meloidogyne* spp. in tomato production systems under greenhouse. It also presents useful information on the use of different cultivation media and substrates for massive *A. oligosporus* spore concentrates.

Key words: *Arthrobotrys oligosporus*, *Meloidogyne*, biocontrol, root system.

Introduction

Phytoparasitic nematodes are considered one of the greatest threats to agriculture worldwide, with estimated losses exceeding \$100 billion per year¹. Nematodes of the genus *Meloidogyne* are the essential phytoparasitic pathogens from an economic and scientific standpoint^{2,3} as they can affect more than 2,000 species of plants⁴, including cultivars of enormous food importance^{5,6}. Previous studies have described 100 species belonging to the genus *Meloidogyne*⁷, of which *M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria* have been reported in Ecuador^{8,9}, causing damage on more than 25% of the horticultural yield production of the country¹⁰.

The management of *Meloidogyne* spp. is cumbersome as different reproductive strategies allow this species to adapt quickly to a wide range of temperatures and environments¹¹. This group's high diversity and the intensive crop production practices exacerbate their incidence and facilitate their dispersal across fields^{12,13}. These nematodes attack exposed roots by modifying their structure and growth, causing the formation of giant cells due to nutrient uptake¹⁴, which translates, sometimes in a short time, into low yield¹⁵.

With the growing advance of agriculture, chemical pesticides were developed¹⁶ to overcome this problem. However, their frequent use has caused several side effects, affecting soil health by reducing biological activity¹⁷. Another concern about pesticides is the risk they pose to human health, including infections, malignancies, lung inflammation, and others^{18,19}. New regulations regarding the use of these chemicals continue to be enforced by international agencies to help alleviate the negative effect of their use in farmers' lives²⁰. In this regard, the search for alternatives to mitigate the effect of nematodes less environmentally damaging and safer for human use is highly encouraged. Among those, biological control agents are a feasible option for pest management²¹. Examples clearly have shown that controlling agents can reduce root-knot nematode populations and damage levels²² up to 45% compared to un-

controlled conditions²³.

Within the gamma of antagonistic microorganisms, nematophagous fungi outstand this activity on trapping and devouring nematodes using specialized structures that allow them to penetrate the cuticle's nematode to grow inside, utterly killing them^{24,25}. Although their habitat is the soil, these fungi can change their lifestyle from saprophytic to pathogenic once they contact the nematode^{26,27}. Some reported modes of action include the development of specialized traps such as constriction rings, non-constriction rings, adhesive knobs, adhesive nets, and adhesive columns^{28,29}. Some more specialized species, such as *Arthrobotrys oligosporus*, can even produce volatile compounds to attract their prey until the mycelial traps are ready to function correctly, which in some cases, can take more than twelve hours to form³⁰.

A. oligosporus grows in soil, around roots, and animal feces³¹, but found particularly in nematode-infested soils³². This fungus has significantly reduced pathogen populations³³. It has been extensively studied under laboratory and semi-controlled and open field conditions in tomato production^{34,35}. Thus, the search for new strains of *A. oligosporus* with similar or better nematophagous activity can help develop new products for controlling *Meloidogyne* spp. in Ecuador to improve tomato's local production. This study's objective was to evaluate the predatory activity of *A. oligosporus* strain C-2197 against the root-knot nematode *Meloidogyne* spp.

Methods

A. oligosporus strain C-2197 was isolated from decomposing leaves collected at the site of Santa Rosa, El Oro province (Ecuador), in a forest adjacent to a coffee plantation with limited anthropogenic activity. The samples were collected in plastic bags and taken to the laboratory, where they were placed in humid chambers for fifteen days^{36,37}. After the isolation and

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identification of this fungus using taxonomic keys, monoconidial colonies were obtained for later use. *A. oligosporus* C-2197 was deposited in the Microorganism Culture Collection of the Biotechnology Research Centre of Ecuador (CIBE) with the code CCMCIBE-H481. To maintain the predatory activity of our strain, the colonies were kept in cornmeal agar media (CMA)³⁸.

For this study, the initial population of *Meloidogyne* spp. was obtained from tomato plants of the Granja Agrícola Experimental (GEA) located in the Escuela Superior Politécnica del Litoral (ESPOL). To maintain the nematode population, hybrid ABM-135 tomato plants were infected and kept in the greenhouse.

The growth of *A. oligosporus* C-2197 was evaluated in six different culture media: water agar, v-8 juice agar, corn meal agar (CMA), carrot agar, cornmeal + carrot agar, and oat agar. Radial growth was measured every 24 hours up to eight days. During this time, the Petri dishes were incubated at 28°C. Then, spore counts were performed in solutions obtained after scraping the colonies' surfaces with 5 ml of sterile water. The number of spores was calculated using a Neubauer camera. The experiment included five replicates per treatment.

To evaluate the predatory effect of *A. oligosporus* C-2197 under laboratory conditions, ten egg masses of *Meloidogyne* spp. were placed in Petri dishes with water agar. After 24 hours from the juvenile nematodes' appearance, 1 ml of spore solution of *A. oligosporus* C-2197, previously adjusted to 1×10^6 cfu/ml, was poured into each Petri dish. This trial included the evaluation of ten replicas (Petri dishes) placed at ambient temperature. After 24 hours of incubation, the number of live, dead, static, parasitized, and non-parasitized nematodes were evaluated.

To assess the effect of *A. oligosporus* C-2197 under greenhouse conditions, three-week old ABM-135 hybrid tomato plants were inoculated with ten egg masses of *Meloidogyne* spp. four days before placing the spore suspensions (1×10^6 cfu/ml) of the biocontrol. For this experiment, spore suspensions were obtained from colonies growing on a natural fiber substrate. The experiment was conducted following a completely randomized block design with four plants per treatment with four replicates (N=48), including T1 - *A. oligosporus* (AOL); T2 - Chemical control (NQ), and T3 - Water (CONTROL). Nematode root infection was evaluated by counting the number of egg masses and determining root-knot damage³⁹. Response variables included plant height, stem diameter, fresh and dry weight of the plant, fresh and dry weight of the roots.

Finally, the effect of *A. oligosporus* C-2197 was also evaluated in small field plots. Tomato plants previously infected with ten egg masses of *Meloidogyne* spp. and then planted in

the field were treated with 1 L of a spore solution (1×10^6 cfu/ml) of *A. oligosporus* C-2197 sprayed directly on a "Bio-cover" (made of banana fiber woven with straw from rice) (Figure. 1 A-B). The experiment included plants treated with a commercial product and negative control for proper comparison. In total, 10 plants were evaluated per treatment for six weeks following a completely randomized block design. Response variables included plant height, number of flowers and fruits.

Results

The radial growth experiment showed that *A. oligosporus* C-2197 has a differential growth response in all media evaluated (Figure. 2A). The fungus thoroughly colonized the Petri dishes' surface, in some cases, from the fifth day. Of the six media evaluated, the best results were observed with oatmeal and cornmeal agar + carrot agar, however, no significant differences were found (Figure 2B). Sporulation of *A. oligosporus* C-2197 was highly increased with significant differences ($p < 0.05$) in v8 juice agar and corn meal agar CMA. Spore count in these treatments was higher than 1×10^6 cfu/ml after nine days of mycelium growth. In contrast, the fungus produced fewer spores on water agar and carrot agar (Figure 2C).

The *in vitro* confrontation test revealed that *A. oligosporus* C-2197 had a control efficiency of 72.31% over the total number of nematodes exposed to the fungus. This percentage included dead and static nematodes (Figure. 3 A-B).

Control activity against *Meloidogyne* spp. was also observed in the greenhouse assay. Plants treated with *A. oligosporus* showed better root development than controls (Figure. 4) and significant differences in nematode egg mass reduction, with an effectiveness of 79%, compared to the negative control (Table 1). The plants' root health treated with *A. oligosporus* also correlated with the increase of plant height at the end of the experiment, which differed significantly from the controls (Figure. 5). Levels of root nematode infestation also differed among treatments. Plants treated with *A. oligosporus* and the commercial product showed less root-knot damage (values between 0-2) than the control (values 3-4), according to the qualitative scale used.

Field experiments also showed plants treated with *A. oligosporus* had better development (20 cm above control plants) (Table 2); however, there were no significant differences regarding the number of flowers and fruits produced by plants on each treatment.

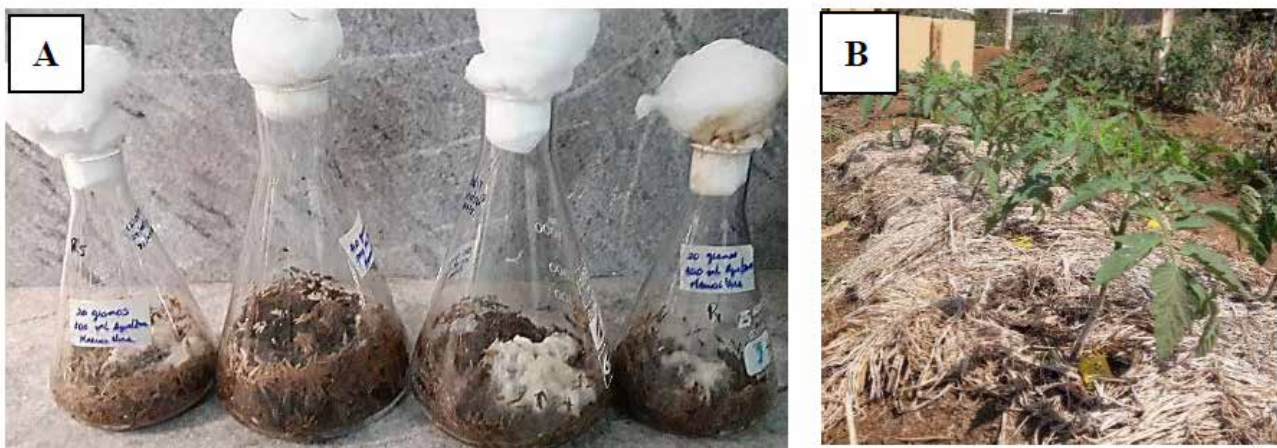


Figure 1. (A) Inoculum production of *A. oligosporus* C-2197 in flasks under controlled conditions. (B) "Bio-cover" inoculated with *A. oligosporus* C-2197 surrounding hybrid tomato plants ABM 135 after 2 weeks of transplant in field plots.

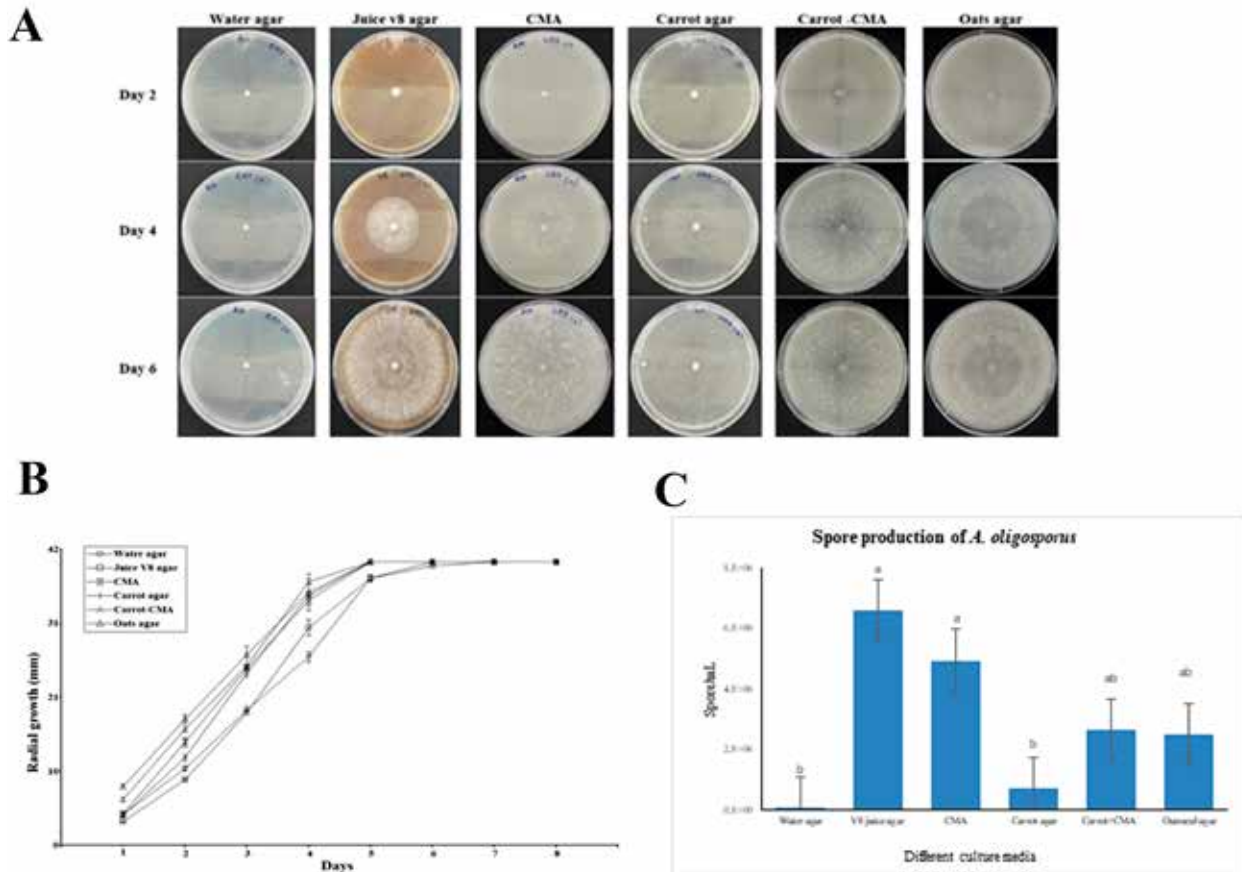


Figure 2. (A) Comparison of the growth of *A. oligosporus* C-2197 in different media, evaluated on the second, fourth and sixth day. (B) Radial growth of *A. oligosporus* C-2197 cultivated in six different media cultures: water agar, v-8 juice agar, corn meal agar CMA, carrot agar, cornmeal + carrot agar, and oatmeal agar. (C) Spore production of *A. oligosporus* C-2197 on different culture media.

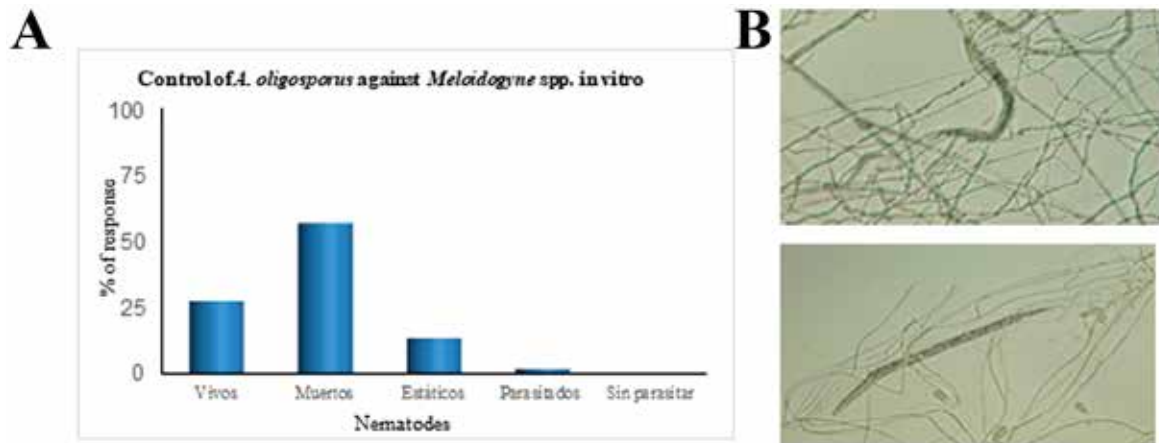


Figure 3. (A) Response percentage of the application of *A. oligosporus* against the nematode *Meloidogyne* spp. under *in vitro* conditions. (B) Nematodes trapped in three-dimensional webs of *A. oligosporus* C-2197

Treatment	Parameters						
	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Root height (cm)	Numbers of egg mass/plant	Gall index (0-10) ^A
1 AOL	136,40 ± 6,12 ^A	21,73 ± 0,92 ^A	22,40 ± 1,73 ^A	2,73 ± 0,19 ^A	42,13 ± 1,78 ^A	23,93 ± 2,95 ^A	1,0 ± 0,1 ^A
2 NQ	78,38 ± 5,93 ^B	11,06 ± 0,89 ^B	15,44 ± 1,68 ^B	2,13 ± 0,19 ^{AB}	38,56 ± 1,72 ^{AB}	42,75 ± 2,86 ^A	1,8 ± 0,1 ^A
3 CONTROL	68,44 ± 5,93 ^B	10,25 ± 0,89 ^B	16,38 ± 1,68 ^B	1,88 ± 0,19 ^B	37,38 ± 1,72 ^B	113,63 ± 2,86 ^B	3,8 ± 0,2 ^B

^A Root-knot damage index on the scale from 0 = no galls to 10 = dead plant according to Bridge and Page (1980). Different letters in the same row indicate statistically significant differences according to Tukey test ($p < 0.05$); ± standard error.

Table 1. Effect of *A. oligosporus* on tomato plants inoculated with the root-knot nematode *Meloidogyne* spp. in greenhouse conditions.

Time	Treatment	Variables					
		Height (cm)		Number of flowers		Number of fruits	
Week 1	T1-AOL	81,61 ±	1,68 ^A	1,40 ±	0,31 ^A	0,17 ±	0,20 ^A
	T2-NQ	71,31 ±	1,62 ^B	1,94 ±	0,30 ^A	0,50 ±	0,20 ^A
	T3-CONTROL	76,13 ±	1,62 ^{AB}	2,19 ±	0,30 ^A	0,75 ±	0,20 ^A
Week 2	T1-AOL	98,63 ±	2,89 ^A	2,13 ±	0,38 ^A	0,44 ±	0,23 ^B
	T2-NQ	85,56 ±	2,80 ^B	3,00 ±	0,36 ^A	1,06 ±	0,22 ^{AB}
	T3-CONTROL	92,06 ±	2,80 ^{AB}	2,88 ±	0,36 ^A	1,38 ±	0,22 ^A
Week 3	T1-AOL	105,62 ±	3,39 ^A	1,71 ±	0,51 ^A	1,37 ±	0,23 ^A
	T2-NQ	92,38 ±	3,28 ^B	2,81 ±	0,50 ^A	2,13 ±	0,22 ^A
	T3-CONTROL	97,19 ±	3,28 ^{AB}	2,50 ±	0,50 ^A	1,81 ±	0,22 ^A
Week 4	T1-AOL	112,55 ±	3,66 ^A	2,14 ±	0,35 ^A	2,42 ±	0,25 ^{AB}
	T2-NQ	96,31 ±	3,54 ^B	2,56 ±	0,34 ^A	3,13 ±	0,24 ^A
	T3-CONTROL	100,69 ±	3,54 ^{AB}	2,38 ±	0,34 ^A	2,19 ±	0,24 ^B
Week 5	T1-AOL	120,67 ±	3,87 ^A	1,35 ±	0,34 ^A	3,36 ±	0,36 ^A
	T2-NQ	99,25 ±	3,74 ^B	1,06 ±	0,33 ^A	3,75 ±	0,35 ^A
	T3-CONTROL	103,63 ±	3,74 ^B	1,88 ±	0,33 ^A	2,63 ±	0,35 ^A
Week 6	T1-AOL	127,44 ±	4,07 ^A	1,69 ±	0,36 ^A	3,63 ±	0,38 ^A
	T2-NQ	104,88 ±	3,93 ^B	0,63 ±	0,35 ^A	4,25 ±	0,37 ^A
	T3-CONTROL	109,00 ±	3,93 ^B	1,31 ±	0,35 ^A	3,13 ±	0,37 ^A

Different letters in the same row indicate statistically significant differences according to Tukey test ($p < 0.05$); ± standard error; * control

Table 2. Evaluation of *A. oligosporus* C-2197 on some physiological and agronomic traits of tomato (*Solanum lycopersicum*) plants hybrid ABM 135

Discussion and Conclusions

In the present study, laboratory, greenhouse, and field tests were conducted to assess the activity of *A. oligosporus* C-2197 as a biocontrol for *Meloidogyne* spp. The in vitro tests showed that *A. oligosporus* C-2197 grew differently depending on the type of medium used, similar to previous reports^{40,41}. Our results are also consistent with previous work on the predatory activity of *A. oligosporus* controlling second instar juveniles of *Meloidogyne* spp. This fungus specializes in the formation of capture nets and demonstrates the ability to attract nematodes⁴²⁻⁴⁵ and the production of toxins that paralyze the movement of juveniles⁴⁶.

Greenhouse tests confirmed the control activity of *A. oligosporus* C-2197 on the nematode population studied. This strain also showed growth-promoting activity for root development and leaf area. These data coincide with previous findings

also reporting growing stimulus of plants after been treated with *A. oligosporus*, increasing production on tomato, rice, and carrot crops^{23,34,47-49}. Laboratory results showed that *A. oligosporus* C-2197 could develop well in substrates with high carbon and nitrogen ratios related to its saprophytic lifestyle, a necessary feature to establish in the soil outperform other saprophytic competitors^{50,51}. This characteristic facilitates the search for cheap substrates that can be implemented for massive scale production of a commercial product based on this strain⁵².

In the present study, the application of *A. oligosporus* to the soil did not significantly affect nematode populations. More extensive field trials with *A. oligosporus* C-2197 are needed, as the "Bio-cover" may have affected the control of the fungus' efficacy. Evidence shows the efficacy of *A. oligosporus* to trap nematodes can vary when conditions are too variable⁵³⁻⁵⁵.

In conclusion, it was demonstrated that *A. oligosporus*

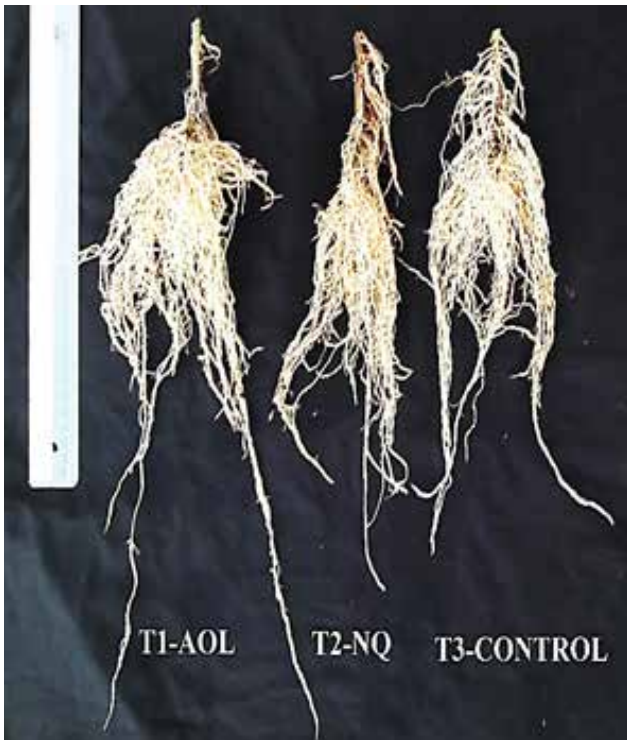


Figure 4. Greenhouse tests results. Evaluation of the root system.

C-2197 showed significant efficacy in controlling nematodes in the laboratory and greenhouse, capable of reducing the target nematode population. Besides, *A. oligosporus* C-2197 had growth-promoting activity on tomato plants. This study shows the potential use of *A. oligosporus* C-2197 for *Meloidogyne* spp.'s biocontrol, specifically as a management alternative for tomato production in greenhouse conditions.

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Figure 5. Foliar development of tomato plants inoculated with *Meloidogyne* spp. and treated with *A. oligosporus* (center), commercial product (right), and control (left).

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