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Morpho-Molecular Characterization of *Ditylenchus dipsaci* and Alternatives for Its Management in Green Onion *Allium fistulosum* Crops from Colombia

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Abstract

Green onion is one of the most consumed vegetables in Colombia. However, its production is negatively affected by the nematode *Ditylenchus*. To determine the species of the nematode, as well as to propose management strategies for its control, in the present study the morphological, morphometric, and molecular characterization of the phytonematode was carried out and was used to evaluate the effect of immersion of the propagation material in water hot, application of a commercial strain of *Purpureocillium lilacinum* and the application of an agrochemical with insecticidal-nematicidal action to control the phytosanitary problem under field conditions. The morphological and morphometric characteristics of the nematode were similar to those reported for the type and reference populations of *D. dipsaci*. Based on sequences of the D2-D3 segment and Internal Transcribed Spacer-ITS of the rRNA, the presence of *D. dipsaci* in green onion crops in Colombia was confirmed. The application of *P. lilacinum* statistically showed an efficient control of *D. dipsaci*, at the same time that presented the highest yield, in relation to the other evaluated treatments ($P \leq 0.05$).

Keywords

Onion Rot, Stem and Bulb Nematode, D2-D3, ITS, *Purpureocillium lilacinum*

1. Introduction

The green onion is one of the four main vegetables grown in Colombia with a production of 340,943 tons/year, being the main producers Boyacá, Santander, Nariño, Antioquía, Norte de Santander, Magdalena, Risaralda and Valle del Cauca states with 95% of national production [1]. The production of this vegetable is commercialized in local markets because it is one of those with the highest demand and per capita consumption with 8.32 kg/year, after tomatoes with 9.4 kg/year [2].

The high national demand for green onions is because it is used mainly as a seasoning for the preparation of different foods, due to their pungent smell and flavor produced by the sulfur compounds present in the tissues in the form of non-protein amino acids, which makes it a special ingredient [3]. In addition to its seasoning properties, green onion is used for medicinal purposes to reduce cholesterol and blood sugar and prevent strokes, obesity, and cancer, due to the mineral content (calcium, iron, magnesium, phosphorus, sodium, zinc, and potassium), vitamins (A, B6, B12, C, D, E, and K) and substances such as quercetin, which is a flavonoid [4].

Unfortunately, green onion production is negatively affected by different phytosanitary problems, one of the most limiting being the disease known as onion rot, caused by *Ditylenchus dipsaci*, an endoparasitic nematode that mainly attacks aerial parts (stems, leaves, and flowers) of a wide range of plants in temperate regions. Almost 500 plant species of 40 families are registered as host of *D. dipsaci* including oats, potato, maize, sugar beet, *Phaseolus* and *Vicia* bean, pea and carrot [5] [6] [7].

In green onion crops, the nematode can attack the plants in any of the phenological stages, but when it occurs in the early stages of growth and development, the effects are more severe [3]. In plants infected with the nematode, the first symptoms are observed as the drying of the leaves apex, which later turns yellow and deformed until they completely wither. Other symptoms in plants include a reduction in the number and height of stems, as well as cracks at the base of the stem that cause the disintegration of membranes and consequent ostensible loss of the root system [8]. The attack by *D. dipsaci* can favor the entry of fungi and bacteria into the plant, which gives a dark color to the lesions caused by the nematode [3] [9] [10].

The economic thresholds of the phytonematode are very low and in population densities of 0.2 - 10 individuals of *D. dipsaci*/100g soil significant losses occur in onion crops [10] [11] [12] [13] [14]. In susceptible onion materials, a positive relationship has been recorded between the initial and final population levels of *D. dipsaci* [14], with plant death in the presence of 2500 individuals of the nematode [8]. Yavuzaslanoğlu *et al.* registered a negative relationship among the population level of *D. dipsaci* and the onion bulb yield, diameter and length in a susceptible cultivar, with production losses from 25 nematodes/100g soil [14].

Between 10 - 30 cuts or harvests of green onion are made during the same

crop cycle in lots free of *D. dipsaci* in Colombia. However, in fields highly infested with the nematode, up to three cuts are currently made, presenting losses of more than 60% of the production [3] [15] [16] [17]. Different practices have been considered to favor the dispersion and population increase of the nematode in the main producing areas, including monoculture, no crop rotation or intercropping with other species, use of contaminated propagation material from infested lots, inadequate handling of crop residues, excessive use of raw or non-composted chicken manure (40 - 80 tons per hectare per year), excessive irrigation with application frequencies of eight days, inadequate or non-existent drainage system, inadequate or irrational use of pesticides and ignorance of the cause of the disease [16].

Although *D. dipsaci* has been reported to affect green onion crops in different producing areas of Colombia, including Tenerife (Valle del Cauca), Silvia (Cauca), and Aquitania (Boyacá), records of the nematode lack taxonomic information with morphometric and molecular support [8] [9] [15]. Therefore, it is necessary to confirm through integrative taxonomy the presence of the nematode *D. dipsaci* in Colombian green onion crops, in addition to evaluating different strategies for disease management. In this sense, the objectives of this study were: 1) To characterize morphologically and molecularly populations of the genus *Ditylenchus* associated with green onion crops in Colombia, and 2) To evaluate the effect of different control methods for the sustainable management of the nematode in green onion crops in Colombia.

2. Materials and Methods

2.1. Sampling and Morphological and Morphometric Identification of Phytonematodes

In two green onion production systems in Colombia, one located in Tenerife (Valle del Cauca Department) and the other in Aquitania (Boyacá Department), field inspections were carried out to detect plants with rot characteristics, according to the symptoms reported for the disease in the literature and by the local farmers including reduced aerial development, twisted, chlorotic and withered leaves. Photographic record of the disease was made and subsamples of symptomatic tissue were collected to form a composite sample of approximately 1 kg. The samples were carefully packed, labeled, and transported to the molecular biology laboratory of Universidad Nacional de Colombia, Palmira campus for further analysis.

Plant parasitic nematodes present in the collected samples were extracted by the oxygenation method, relaxed, and killed by immersion in a water bath at 65˚C for 4 min. Subsequently, the nematodes were fixed in 2% formalin and semi-permanent preparations were made on slides [18]. In a compound microscope equipped with image analyzer software (Carls Ziss), morphological and morphometric data were recorded to the extracted nematodes for its identification to species level based in the comparation of measurements among the ana-

lyzed and type populations [19] [20] [21]. The photographic record of the nematodes was carried out in a microscope equipped with Differential Interference Contrast-DIC, Nikon Eclipse model 80i brand, and Nis elements image capture system.

2.2. Molecular Identification of Phytonematodes

DNA extraction from phytoparasitic nematodes was carried out following the proteinase K protocol [22]. For this purpose, a single specimen was cut and transferred to a tube with 15uL of lysis buffer (50 Mm KCl, 10 Mm Tris pH8.3; 15 Mm MgCl2; 0.5% Triton x-100; 4.5% Tween 20; 0.09% proteinase K). Subsequently, the tube was incubated at −80˚C (15 min.), 65˚C (1 hr.), and 95˚C (15 min.). Finally, the tube was centrifuged (1 min. at 16,000 g) and stored at −20˚C. PCR was performed to amplify the ITS region of the rRNA using the primer TW81 forward (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 reverse (5'-ATATGCTTAAGTTCAGCGGGT-3') [23]. Additionally, the D2-D3 segment of the large rRNA subunit (28S) was amplified using the primer D2A forward (5'-ACAAGTACCGTGAGGG AAAGTTG-3') and D3B reverse (5'-. CCTCGGA AGG AACCAGCTACTA-3') [24]. These genome regions are reported to be informative and useful to the molecular identification of plant-parasitic nematodes included *Ditylenchus* species due respectively to the rate high of nucleotide substitution and high inter-specific variability. For both regions, the PCR conditions were initial denaturation for 2 min. at 94˚C, followed by 40 cycles of 45 sec. at 94˚C, 45 sec. at 55˚C, 1 min. at 72˚C and a final extension of 10 min. at 72˚C. PCR products were sequenced in both directions by Bioneer Corporation, South Korea.

2.3. Phylogenetic Analysis of Phytonematodes

The obtained concensus sequences were edited using the software Geneius [25]. After the sequences were refined, their identity was confirmed by comparison in the GenBank database, using the BLASTn tool (http://www.ncbi.nlm.nih.gov/BLAST). The sequences obtained in the present study and others downloaded from the GenBank with accession numbers referenced in **Table 1**, were manually aligned using the MEGA 6 program [26]. Based on the matrix obtained for each gene, the best nucleotide substitution model was determined, taking into account the Bayesian Information Criterion (BIC) and using the Model Generator v.0.851 program [27]. For both genes, phylogenetic trees were constructed using the maximum likelihood (ML) method, along with the 2-parameter Kimura model with gamma distribution, and the reliability of internal nodes was determined using the method with 1000 interactions. The species *Bursaphelenchus xylophilus* (JQ743665) was used as the outgroup of the phylogenetic tree corresponding to the ITS region; while the species *Sphaerularia vespae* (AV300596) was used as the outgroup of the phylogenetic tree based on the D2-D3 segment.

Table 1. Information on the ITS and D2-D3 rRNA sequences downloaded from GenBank and obtained in the present study.

Species name	Host	Location	ITS accession number	Accession Number D2-D3	Reference
D. dipsaci	*Allium fistulosum*	Colombia	OQ718817; OQ718818; OQ718819; OQ718820	OQ718928	Present study
D. dipsaci	*Vicia faba*	Spain	HQ219249	-	[28]
D. dipsaci	*Allium sativum*	Czeck Republic	DQ452958	-	[29]
D. dipsaci	*Allium cepa*	Italy	AY574290	-	[13]
D. dipsaci	*Allium sativum*	Canada	KJ567153	-	[30]
D. dipsaci	*Allium sativum*	Czeck Republic	-	FJ707361	[31]
D. dipsaci	*Allium sativum*	Yemen	-	JF327761	Unpublished
D. dipsaci	Bean	Russia	-	HQ219226	[28]
D. weischeri	*Cirsium setosum*	Russia	AF396322	-	[32]
D. weischeri	*Cirsium arvense*	Canada	KJ567155	-	[30]
D. weischeri	*Cirsium arvense*	Canada	-	MG551903; MG551907	[33]
D. oncogenus	*Sonchus oleraceus*	Italy	KF612016	KF612015	[34]
D. laurae	*Potamogeton perfoliatus*	Poland	KX389268	-	[35]
D. gigas	*Vicia faba*	United Kingdom	AY574284	-	[13]
D. gigas	*Vicia faba*	Italy	HQ219231; HQ219232	HQ219216; HQ219217	[28]
D. askenasyi	*Calliergon* sp.	Estonia	AF396336	-	[32]
D. askenasyi	*Calliergonella cuspidata*	Estonia	AF396337	-	[32]
D. persicus	Grapevine	Iran	KX463286	KX463285	[36]
D. destructor	*Solanum tuberosum*	China	EF208212	-	[37]
D. destructor	*Solanum tuberosum*	Czech Republic	GQ469490; GQ469491	-	[38]
D. destructor	sweet potato	China	-	EU400642; EU400633	[39]
D. myceliophagus	Grass	Canada	KJ567156	-	[30]
D. myceliophagus	*Medicago sativa*	Iran	-	MF996705	[40]
D. africanus	Peanut	South Africa	KJ567154	-	[30]
D. arachis	*Arachis hypogaea*	China	JX040545; JN635037	-	[41]
D. halictus	*Halictus sexcinctus*	Germany	EF627047	AY589364	[42]
D. drepanocercus	*Miconia calvescens*	Brazil	-	JQ429772; JQ429773	[43]
D. gallaeformans	*Miconia albicans*	Brazil	-	JQ429769	[44]
D. gallaeformans	*Leandra lacunosa*	Brazil	-	JQ429770	[43]
D. phyllobius	*Solanum elaeagnifolium*	Mexico	-	KT192617; KT192618	[44]
D. terricolus	*Medicago sativa*	Iran	-	MF996706	[40]
D. acutus	*Medicago sativa*	Iran	-	MF996704	[40]

2.4. Evaluation of Nematode Management Strategies

Using a randomized complete block design with four replications, the effect of four treatments (biological, physical, chemical, and absolute control) on the control of the nematode *D. dipsaci* in green onions were evaluated under field conditions. The biological treatment consisted of eight post-sowing applications to the base of the plant (drench) of a commercial strain of the fungus *Purpureocillium lilacinum* (Lilaciplant®), at a 400 g/ha dose. The physical treatment was based on pre-sowing immersion of the seed in hot water at a temperature of 50˚C for 15 minutes to reduce the inoculum level of the parasite. The chemical treatment consisted of eight post-sowing applications to the base of the plant of an insecticide with nematicidal action, with the commercial name Fulminator® (Cipermectrin and Profenofos as active ingredients), at a dosage of 0.25 cc/L. The absolute control plants did not receive any treatment. During the experiment and with a frequency of 15 days, plant tissue samples were taken from each of the experimental units and the population level of the nematode expressed in the number of individuals/10g of tissue was determined. At the end of the crop cycle, in the same experimental units, the yield expressed in tons per hectare was recorded.

2.5. Statistical Analysis

To evaluate the effect of the treatments on the *D. dipsaci* populations, a Type II model—PROCEDURE MIXED—was used where the random component was the blocks. At a probability of 5%, the comparison of treatment averages within each sample was carried out using LSMEANS. Regarding the yield of the *A. fistulosum*, a fixed effects model—PROCEDURE GLM—was used; for the multiple comparisons of treatment averages, the Tukey Test was applied with a significance level of 5%. The Pearson Correlation Analysis technique was used to establish the relationship between the population levels of *D. dipsaci* with the yields of *A. fistulosum*. For the analysis, the statistical program SAS in version 9.4 was used.

3. Results

3.1. Expression of Symptoms

In green onion crops in the municipalities of Tenerife (Valle del Cauca) and Aquitania (Boyacá) (**Figure 1(A)**), plants with characteristic symptoms of the disease called onion rot were observed. Severely affected plants were characterized by reduced aerial development, twisted, chlorotic and withered leaves (**Figure 1(B)**). At the level of the stems, the diseased plants presented external and internal necrosis and swelling at the base (**Figure 1(C)**, **Figure 1(D)**). Some plants with limited aerial development presented stems with rot (**Figure 1(E)**).

3.2. Morphological and Morphometric Characterization of Phytonematodes

Nematodes identified in the genus *Ditylenchus*, were extracted from the tissue of

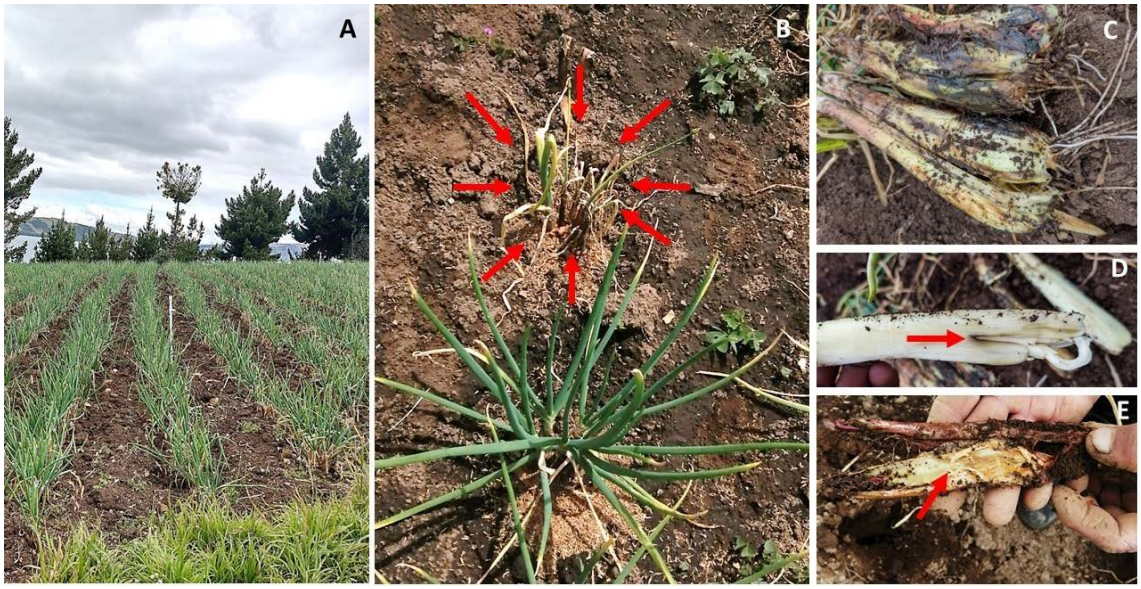

Figure 1. Onion crop with expression of symptoms caused by *D. dipsaci* (A) Green onion crop system in Aquitania-Boyacá, Colombia; (B) Onion plant with rot symptoms; (C) Onion plants with symptoms of external rot; (D) Onion plant with symptoms of internal rot; (E) Onion plant with advanced rot symptoms. The red arrows indicate the appearance of the plant and tissues affected by the phytonematode.

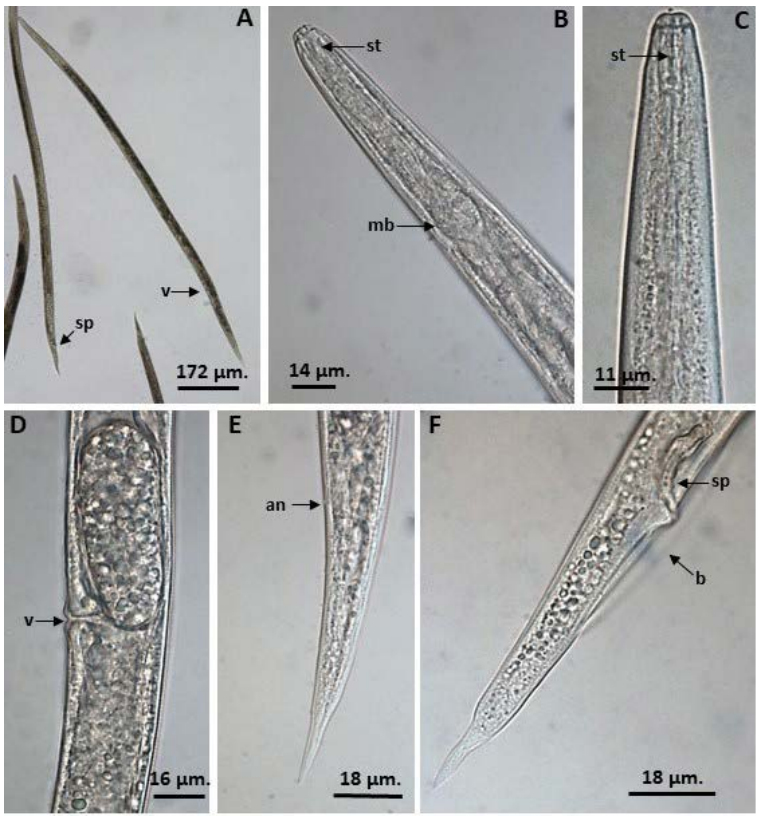

Figure 2. Microscope images with differential contrast of Interference-DIC of *Ditylenchus dipsaci*. (A) Body of a female and a male; (B) and (C) Anterior region of the body of the nematode; (D) Posterior vulvar region of the body of a female; (E) Posterior region of the body of a female at the level of the anus; (F) Posterior region of the body of a male at level of the cloaca. v = vulva, sp = spicule, st = stylet, mb = median bulb, an = anus and b = bursa.

diseased plants in both study areas. The females and males of the nematode were characterized by presenting an almost straight body in a state of rest (**Figure 2(A)**), a labial region slightly flattened and slightly displaced with the contour of the body, short stylet with round basal knobs, medium bulb developed (**Figure 2(B)** and **Figure 2(C)**) and conical tail shape with a pointed end (**Figure 2(E)**). In females, the vulva was located in the posterior part of the body, approximately 80% (**Figure 2(D)**). For their part, the males presented a bursa that extended from the anterior end of the spicule to three-quarters of the tail. The morphological characteristics and morphometric data recorded for these populations agree with those reported for the type and reference populations of *D. dipsaci* (**Table 2**).

3.3. Molecular Characterization of Phytonematodes

Four consensus sequences of the ITS region of the rRNA and one of the D2-D3 segments of the rRNA were generated in the present study. The sequences corresponding to ITS showed a high similarity, between 99.6 and 99.7%, with reference sequences of the *D. dipsaci* species from different hosts (KU179474 from *Allium sativum*, MG384731 from garlic, MK292125 from *French iris*, GQ469496 from *Plantago lanceolata*, DQ452957 of *Chicorium inthybus*). On the other hand, the sequence corresponding to segment D2-D3 presented a 100% similarity with reference sequences of *D. dipsaci* from different hosts (JF327759 from *Allium sativum*, MK292125 from *French iris*, HQ219220 from *Pisum sativum*).

3.4. Bioinformatic Analysis

The alignment based on the ITS region comprised a total of 28 taxa, with a total of 1465 characters including gaps, in which 581 were conserved, 648 variables, and 356 were parsimonious-informative. The alignment of the D2-D3 region included 23 taxa with a total of 883 characters, 400 conserved, 440 variable, and 318 parsimonious.

The phylogenetic analysis for the ITS region shows that the sequences obtained in this research grouped in the same clade with reference sequences of the *D. dipsaci* species, with Bootstrap support of 97% (**Figure 3**). Similarly, the D2-D3 sequence obtained in this study grouped in the same clade with reference sequences from *D. dipsaci* with 99% Bootstrap support (**Figure 4**). In both phylogenetic trees, the clade corresponding to *D. dipsaci* separated from other species of the same genus such as *D. gigas* and *D. destructor*.

3.5. Evaluation of Phytonematode Management Strategies

The most efficient treatments in the management of *D. dipsaci* were biological control with the fungus *P. lilacinum* and chemical control with the insecticide-nematicide, with population levels of 8 individuals of *D. dipsaci*/10g of fresh tissue at the end of the experiment. On the contrary, the treatments with the highest population levels were the absolute control treatment and the physical

Table 2. Morphometric data of studied and reference populations of *Ditylenchus dipsaci*.

Characters	Tenerife (Valle del Cauca, Colombia) Present study (n = 10)	Aquitania (Boyacá, Colombia) Present study (n = 10)	[19] [20] [21]	[45] n = 10	[46] n = 28
L	1349.7 ± 154.6 (1184.0 - 1490.0)	1399.5 ± 45.4 (1315.8 - 1454.8)	1000.0 - 1700.0	1190.4 ± 123.6 (1056.7 - 1418.0)	910 ± 120 (780 - 1280)
a	43.6 ± 3.6 (37.8 - 50.5)	38.5 ± 2.1 (36.0 - 41.7)	30.2 - 64.0	38.7 ± 3.2 (33.4 - 46.4)	44.0 ± 3.4 (37.6 - 50.3)
b	6.9 ± 0.5 (6.0 - 7.5)	6.7 ± 0.2 (6.3 - 7.0)	6.0 - 8.8	7.0 ± 0.7 (6.0 - 8.4)	6.2 ± 0.4 (5.5 - 6.7)
c	15.1 ± 1.2 (12.8 - 16.0)	15.4 ± 0.6 (14.5 - 16.2)	13.3 - 19.5	13.3 ± 1.0 (11.1 - 15.3)	15.1 ± 1.5 (12.5 - 18.4)
c′	4.5 ± 2.0 (5.5 - 5.5)	4.6 ± 0.2 (4.2 - 4.8)	3.0 - 6.0	-	5.1 ± 0.6 (4.1 - 6.5)
V	82.5 ± 0.8 (81.2 - 83.9)	80.8 ± 1.0 (79.8 - 82.8)	79.0 - 86.0	80.0 ± 1.2 (78.9 - 82.2)	79.5 ± 1.8 (75.0 - 82.5)
Lip region height	2.4 ± 0.2 (2.1 - 2.8)	2.6 ± 0.3 (2.3 - 3.2)	-	-	-
Lip region diameter	7.6 ± 0.3 (7.2 - 8.1)	7.7 ± 0.3 (7.1 - 8.1)	-	-	-
Stylet length	11.0 ± 0.7 (10.3 - 12.0)	10.8 ± 0.1 (10.6 - 10.9)	10.0 - 13.0	10.1 ± 0.5 (9.5 - 10.8)	9.8 ± 0.3 (9.4 - 10.5)
Median bulb length	21.0 ± 2.9 (17.2 - 25.9)	22.4 ± 2.9 (14.8 - 24.7)	-	-	-
Median bulb width	13.5 ± 1.5 (10.7 - 15.7)	14.3 ± 1.1 (11.8 - 16.1)	-	-	-
Pharyngeal gland length	62.2 ± 13.8 (41.4 - 81.8)	67.0 ± 7.6 (54.3 - 78.8)	-	-	-
Pharyngeal gland width	17.6 ± 4.1 (12.2 - 22.1)	20.9 ± 1.4 (17.9 - 22.3)	-	-	-
Maximum body diameter	31.8 ± 2.0 (28.9 - 34.9)	36.4 ± 2.2 (33.1 - 39.4)	-	30.8 ± 2.2 (28.0 - 34.6)	-
Pharynx length	199.1 ± 12.2 (187.2 - 224.9)	208.6 ± 7.9 (198.2 - 224.3)	-	-	-
Vulva to anus distance	186.1 ± 48.9 (130.4 - 253.0)	196.1 ± 28.5 (174.5 - 254.1)	-	143.6 ± 23.4 (94.2 - 176.4)	-
Post uterine sac (PUS)	30.6 ± 4.2 (21.4 - 36.0)	43.1 ± 12.0 (21.5 - 68.9)	-	63.9 ± 11.5 (46.9 - 82.5)	-
Anal body diameter	17.5 ± 0.8 (16.7 - 18.7)	20.0 ± 0.9 (18.8 - 21.7)	-	-	-
Tail length	92.0 ± 1.8 (88.3 - 93.1)	90.8 ± 3.4 (87.9 - 96.5)	95.0 - 105.0	90.1 ± 12.4 (77.3 - 111.1)	60.4 ± 6.5 (50.5 - 72.8)

Note: Measurements in µm; mean ± s.d. (range).

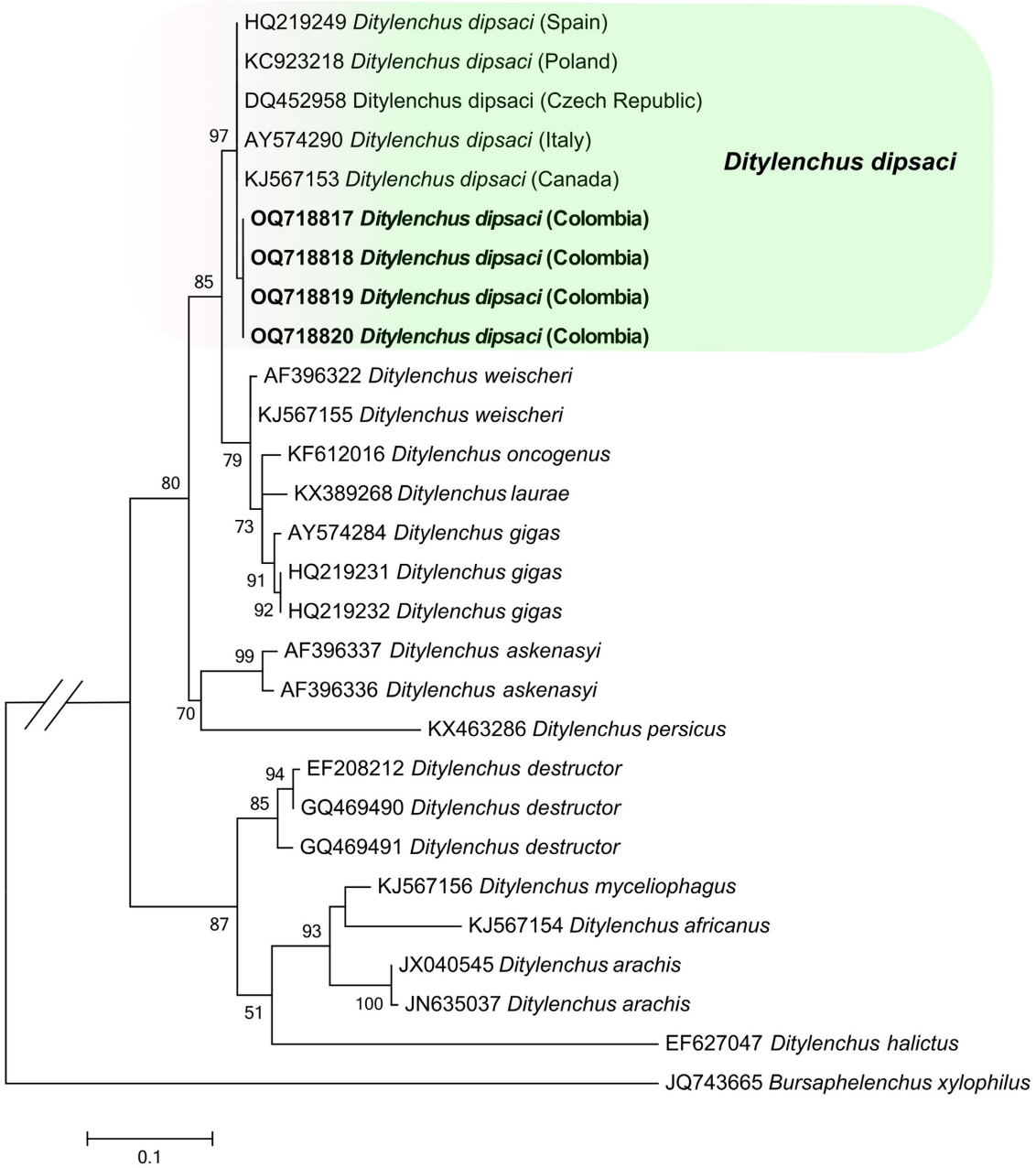

Figure 3. Phylogenetic tree obtained by maximum likelihood of the partial ITS region of species of the genus *Ditylenchus*. Specimens corresponding to this work are marked in bold. Numbers above nodes indicate bootstrap values > 70%. *Bursaphelenchus xylophylus* (JQ743665) was used as the outgroup of the tree.

control, respectively with 106 and 78 *D. dipsaci*/10g of fresh tissue at the end of the experiment.

For the same variable, the analysis of averages showed that no statistical differences were recorded between the biological control and chemical control treatments. Among these treatments and the physical control and the absolute control, statistically significant control of the nematode was observed within each of the sampling periods. Although the physical treatment did not show

control over *D. dipsaci*, the population level was lower than that registered in the absolute control (Table 3).

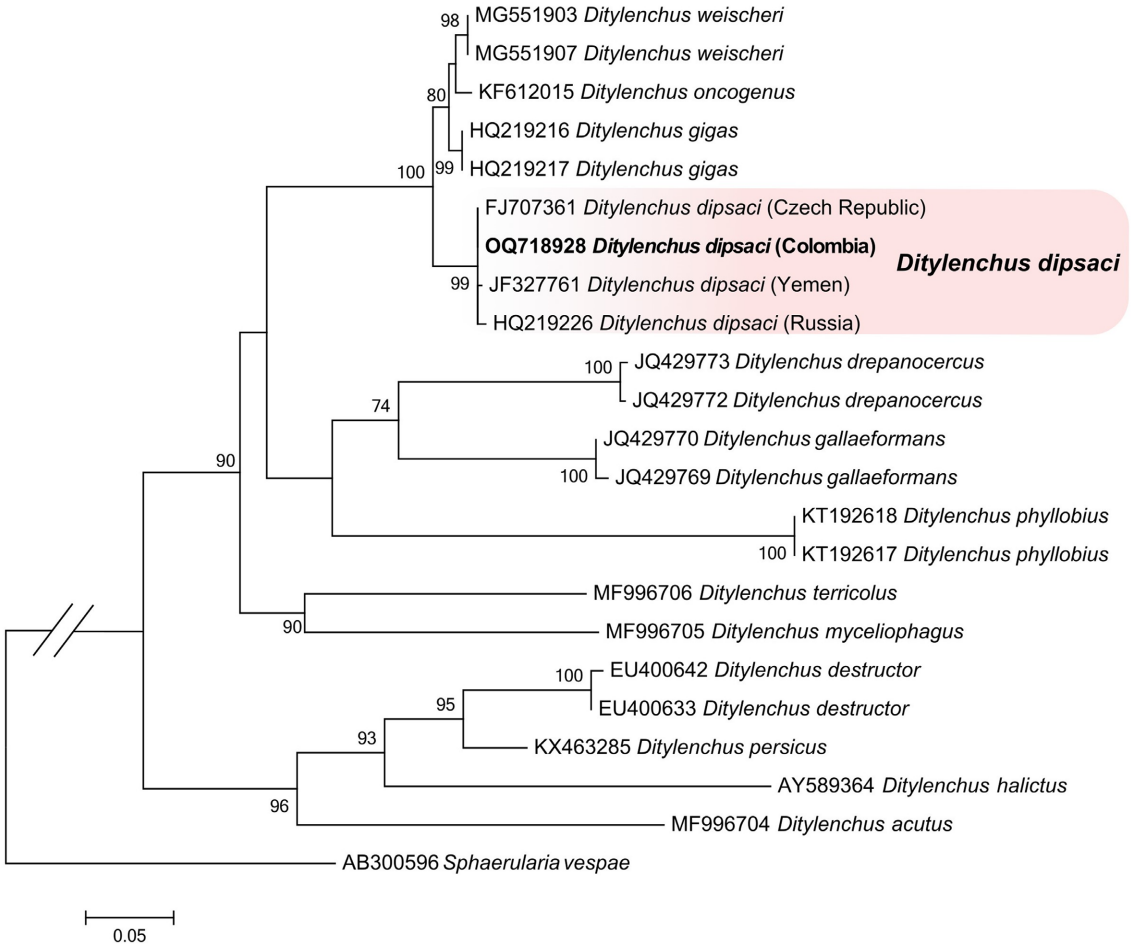

Figure 4. Phylogenetic tree obtained by maximum likelihood of the partial D2-D3 region of species of the genus *Ditylenchus*. Specimens corresponding to this work are marked in bold. Numbers above nodes indicate bootstrap values > 70%. *Sphaerularia vespa* (AB300596) was used as the outgroup of the tree.

Table 3. Analysis of contrasts to compare the population level of *D. dipsaci* between treatments.

Contrast	Sampling time (days after planting) and differences in the number of *D. dipsaci*/10 g of fresh tissue between treatments						
	15	**30**	**45**	**60**	**75**	**90**	**105**
Biological vs Physical	2	2	−20***	−37***	−55***	−65***	−76***
Biological vs Chemical	5**	9***	4	1	−2	−2	−1
Biological vs Control	−6**	−24***	−49***	−61***	−72***	−83***	−104***
Physical vs Chemical	3	8***	24***	38***	52***	63***	75***
Physical vs Control	−8***	−26***	−29***	−24***	−17***	−18***	−27***
Chemical vs Control	−11***	−33***	−52***	−62***	−69***	−81***	−103***

**Statistically significant differences, ***Highly significant statistical differences.

For the yield variable (ton/ha), the results of the analysis of variance show significant differences between treatment averages, with a probability of 0.0031. The highest yield was observed in the biological control treatment with 25.7 ton/ha which differs statistically from the absolute control and the chemical control with 20.8 and 20.0 ton/ha respectively; the lowest yield was presented in the physical control with 13.0 ton/ha. Unlike the other treatments, better root system development was observed in the plants to which the biological control was applied (Figure 5(A)).

Pearson's correlation analysis showed that there is an inverse correlation (r = 0.59) between the population level of *D. dipsaci* and yield in green onion, which is statistically significant (p = 0.034); that is to say that as the population levels of

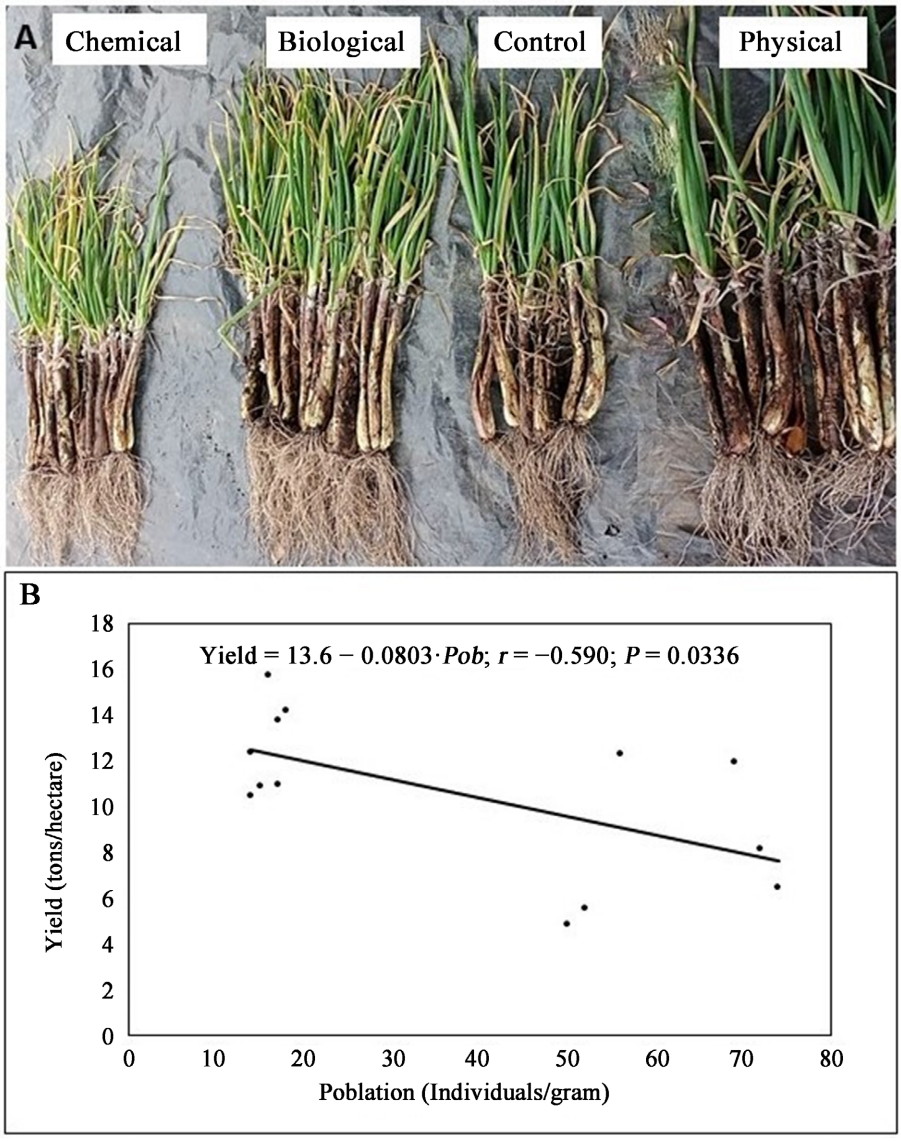

Figure 5. (A) Appearance of green onion plants from the different treatments evaluated in the field experiment, and (B) Correlation analysis between the nematode population level and yield in green onion.

the nematode increase, the yield of the plant species under study decreases (**Figure 5(B)**).

4. Discussion

In both study areas, the affected plants presented twisting, chlorosis, and leaf wilting, as well as stems with internal and external necrosis and swelling at the base. These symptoms agree with those reported in onion crops affected by *D. dipsaci* in Colombia and other regions of the world [8] [9]. From diseased plant material, recovered nematodes were identified as *D. dipsaci* by integrative taxonomy.

The morphological and morphometric characteristics of the specimens were similar to those reported for the type and reference populations of *D. dipsaci* [17] [19] [20] [21] [45]. DNA sequences analysis confirmed Morphological and morphometric identification establishing the presence of *D. dipsaci* in green onion crops from Colombia. This nematode has been reported to affect onion crops in different producing areas of the world, including Chile and Türkiye [14] [17] [47].

An inversely proportional relationship was confirmed in the present study between the population level of *D. dipsaci* and the yield of the green onion, which is consistent with previous reports. Yavuzaslanoğlu *et al.* showed that production in a susceptible bulb onion cultivar was negatively correlated with the initial *D. dipsaci* population [14]. It is known that low population levels of the nematode are required to cause significant losses in onion, which can be up to 60% [10] [11] [12] [14] [17] [47] [48].

Biological management through the application during and post-sowing of *P. lilacinum* showed an effective control of *D. dipsaci* in green onion, allowing to obtain plants with low densities of the nematode and high yields. The efficacy of *P. lilacinum* in the control of phytoparasitic nematodes, as recorded in this research, has been reported in different studies [49] [50] [51] [52].

Different authors mention that *P. lilacinum* mainly parasitizes *Meloidogyne* eggs and other phytoparasitic nematodes, through the secretion of proteases and chitinases that destroy lipids and chitin layers, as well as the integrity of the yolk layer of the egg cover, which allows the penetration of the mycelium of the fungus, subsequent parasitism and hatching inhibition. In addition to proteases and chitinases, metabolites of *P. lilacinum* have been related to its parasitism, including non-ribosomal synthesized pectide antibiotics such as leucinostatins B, D, F, H, L, and T. These action mechanisms are suggested in the biocontrol observed in the present study on *D. dipsaci* [49] [51] [53].

The plants treated with the chemical synthesis product presented a low density of the nematode during the experiment, confirming that the active ingredients Cypermectrin and Profenofos, in addition to insecticides, have nematicidal activity against *D. dipsaci*. The first active ingredient, a synthetic pyrethroid that disrupts neuronal function by activating sodium channels, has been reported to

efficiently control the nematode *Meloidogyne incognita* [54]. For its part, the second active ingredient, a neurotoxin that acts as a cholinesterase inhibitor in the nervous system, has also been reported to decrease the density of phytoparasitic nematodes including *D. destructor* [55] [56].

Although the treatment with the chemical product was effective in controlling *D. dipsaci*, the yield of the treated plants was similar to that of the absolute control, which suggests a phytotoxic effect of Cypermectrin and/or Profenofos, which has been reported in the past and related to the alteration of cell division processes, synthesis of amino acids and nucleic acids, as well as the reduction of the contents of photosynthetic pigments (chlorophyll) in plants [57] [58] [59] [60].

After the absolute control treatment, plants from propagation material treated with hot water at 50˚C for 15 min showed the highest density of the nematode. According to different authors, seed immersion in hot water, as a physical control strategy, can reduce the densities of *D. dipsaci* due to the sensitivity of these microorganisms to high temperatures, at the same time maintaining the viability of the planting material [61]. However, this practice does not completely eradicate nematodes from the seed, allowing individuals that survive treatment to reproduce and reach high densities after some time. Likewise, this control strategy does not protect the propagation material from nematode infection after sowing in infested soils, which explains why the plants of the physical treatment during and at the end of the experiment presented high densities of *D. dipsaci* and consequently a significant reduction in the yield. Similar results were reported by Roberts & Matthews after immersing garlic seed cloves in hot water for the elimination of *D. dipsaci* for 15, 20, 25, and 30 min at 49˚C, finding suppression of the infection by the nematode compared to untreated controls, but infection was significantly higher than in seed immersion treatments in hot water supplemented with formalin, abamectin, or sodium hypochlorite, demonstrating that only partial control of *D. dipsaci* is achieved with the immersion of the seed in hot water without additives [61].

Based on the results of the present study, an alternative for the biological management of *D. dipsaci* in green onion production systems is the use of the nematophagous fungus *P. lilacinum* [49]. In addition to the biocontrol role of *P. lilacinum*, it has been mentioned in the literature for its ability to promote plant growth through the solubilization of phosphorus and production of Indole Acetic Acid-IAA. Some authors mention that *P. lilacinum* can replace the use of chemical nematicides, which are non-specific and non-selective, as well as toxic to vertebrate and invertebrate species [62].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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