Biotecnología Aplicada

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RESEARCH

Evaluation of the bioformulate and raw filtrate of *Purpureocillium* sp. strain *Udea0106 on plant pathogenic n*ematodes in chrysanthemum (*Dendranthema grandiflora*) crops

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ABSTRACT

Plant-parasitic nematodes are the main agricultural pest affecting the floricultural industry of Colombia. Taking into account previous research where strain UdeA0106 of the *Purpureocillium* sp. fungus was shown to exhibit a high nematicidal capacity, the present work evaluated different concentrations of a raw filtrate from this biocontrol agent and performed a field experiment with a bioformulation thereof. The variables evaluated in both cases were the genus and numbers of nematodes in soil and the gall index. Additionally, the field trial evaluated productivity variables such as the number of flower buds, plant weight and height and stem width. All the evaluations were repeated twice, and the existence of statistically significant inter-treatment differences was detected via ANOVA or Kruskal-Wallis tests, using 0.05 as cut off for statistical significance, as well as multiple comparison tests such as Tukey's and kruskalmc, as implemented in R®. The raw filtrate produced a statistically significant decrease in the number of juvenile-stage *Meloidogyne* spp. and *Paratylenchus* spp. nematodes in soil, although it failed to reduce root infestation indexes. The bioformulation from strain UdeA0106 also produced statistically significant decreases in the number of nematodes when compared to control treatments. In the case of the nodulation variable, both the filtrate and the bioformulation of UdeA0106 yielded lower numbers and indexes compared to the chemical products evaluated. For the "weight" productivity variable, the bioformulation field experiment showed differences between treatments with 4.6 g, a desirable aspect in the production of flowers.

Keywords: biocontrol fungus, *Purpureocillium* sp., UdeA0106 strain, nematodes, *Meloidogyne incognita-javanica*, *Paratylenchus* spp., chrysanthemum

RESUMEN

Evaluación del bioformulado y del filtrado crudo de *Purpureocillium* sp. cepa Udea0106 sobre nemátodos fitopatógenos en crisantemo (*Dendranthema grandiflora*). La floricultura, es un sector productivo importante para la exportación en Colombia y son afectados por los nematodos fitopatógenos. El hongo *Purpureocillium* sp. cepa UdeA0106 ha registrado previamente una alta capacidad nematicida, por lo que se evaluó su filtrado crudo en diferentes concentraciones in planta, así como su bioformulado en campo. Las variables evaluadas para ambos casos fueron el género y número de nematodos en suelo, índice de agallamiento y en los ensayos de campo, se evaluaron variables de productividad (número de flores, peso, altura y grosor de las plantas). Todos los ensayos se repitieron dos veces en el tiempo y para el análisis estadístico tanto del filtrado como del bioformulado se realizaron las pruebas de ANOVA (p = 0.05) y de Kruskal-Wallis. Para determinar diferencias entre los tratamientos, se realizaron pruebas de comparación múltiple de Tukey y de comparación múltiple de Kruskal mediante el programa estadístico R®. Los resultados mostraron que en las mayores concentraciones del filtrado se presentó un menor número de juveniles de *Meloidogyne* spp. y *Paratylenchus* spp., sin efecto preventivo a la infestación de raíces. Un comportamiento similar presento el bioformulado de la cepa UdeA0106 evidenciando diferencias estadísticamente significativas entre ésta y los demás tratamientos. Para la variable de nudosidad al aplicar el filtrado y la bioformulación de UdeA0106, se observó un menor número e índice, en comparación de los productos químicos evaluados. Para la variable "peso" de productividad, el experimento del bioformulado en campo presento diferencias entre tratamientos con 4.6 g, aspecto deseable en la producción de flores.

Palabras clave: hongos biocontroladores, *Purpureocillium* sp., cepa UdeA0106, nematodos, *Meloidogyne incognita-javanica*, *Paratylenchus* spp., crisantemos

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Introduction

Chrysanthemum (*Dendrathema grandiflora*) is one of the most important products of the Colombian floricultural industry, representing its third most important export to the US and British markets [1]. In Colombia, chrysanthemums are cultivated mainly in westerly lands of the department of Antioquia, where the most important agricultural pest threatening the production of this crop are plant-parasitic nematodes. The alterations caused by these pests on the radicular roots of the affected plants decrease nutrient absorption, thereby affecting soil anchorage, producing chlorosis and ultimately lowering production yields [2].

Some of the most important genera of plant-parasitic nematodes affecting flowering plants are

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Meloidogyne spp., *Paratylenchus* sp., *Pratylenchus* sp. *Helicotylenchus* sp. and *Trichodorus* sp. These pests are usually managed through proper cultural practices, the use of resistant cultivars and the application of chemical pesticides [2]. However, chemical pesticides suffer from a number of significant disadvantages, as they can be toxic to humans, ecologically damaging and costly from an economic viewpoint.

Clean, environmentally friendly alternatives to chemical pesticides for the control of plant-parasitic nematodes (including *Meloidogyne* spp., the most economically important genus) have been available for years [3-5]. One of these alternatives is the use of biocontrol agents, specifically, the utilization of fungi with the ability of controlling nematode populations through a number of mechanisms that includes direct action through fungal structures (as in the case of endophytic and entrapping species) and indirect action through fungal toxins [5].

Among the fungi employed as biocontrol agents, the genus *Purpureocillium* spp. occupies a prominent position [6]. A particular strain of this genus, UdeA0106, which is kept at the strain collection of the FITOBIOL (Phytopathology and Biotechnology of Fungi) group at the University of Antioquia (Medellín, Colombia), has been shown to parasite nematodes of the *Meloidogyne* spp. and *Paratylenchus* spp. genera under laboratory conditions, on which it exerts a nematicide effect through both structural features [7-9] and compounds present in raw fungal filtrates [8, 10]. Prompted by these results, the University of Antioquia contacted a commercial partner with expertise in the production of bioformulates to develop a production-ready suspension of this fungus, which was evaluated *in vitro* and in a preliminary field study that yielded promising data [8]. Hence, and considering the need for field evaluations under conditions closer to those of floricultural production, the present work has the objective of assessing the nematicidal effect of raw filtrates and commercial bioformulates of strain UdeA0106 on genera affecting the cultivation of chrysanthemums as well as the result of their application on variables relevant for the quality of this floricultural product.

Materials and methods

Experimental work

The laboratory experiments of the present work were performed at the facilities of the FITOBIOL group of the Institute of Biology of the University of Antioquia (Medellín – Colombia), whereas the field evaluation took place during 2015 to 2016 under production conditions in commercial facilities for the cultivation of chrysanthemums, at Miramonte 2 ranch (Aut. Km 39 Vda Belén Marinilla-Colombia). Miramonte 2, which has average precipitation 2000 mm and temperatures of 22 °C, has over 40 hectares of soil dedicated to the production of chrysanthemum flowers for export, mainly to the US market. These soils have relatively high infestation indices for floriculturally relevant plant-parasitic nematodes.

Experimental work took place in two stages. Stage I consisted of the evaluation in soil and in planta of filtrates from strain UdeA0106, and Stage II consisted of the evaluation, under field conditions, of the bioformulate obtained from this strain.

Prior to the preparation of the filtrate and the bioformulate, strain UdeA0106 was activated from a sporulated, pure culture grown on *Meloidogyne* spp. eggs.

Stage I: Evaluation of the filtrate

The raw filtrate (liquid culture medium that is filtered after vegetative growth of the fungus has taken place) was prepared following a modification of a previously published method [9], reducing the incubation time from 7 to 5 days and the filtrate was centrifuged at 5000 rpm for 20 min. After centrifuging the cultures, the resulting supernatant was filtered through a 0.45 µm pore-size cellulose membrane (Millipore, USA) and then through a 0.20 µm pore-size cellulose membrane (Millipore, USA).

Nematode control in soil and in planta

The nematicidal effect of the UdeA0106 filtrate was evaluated in three different experiments. In the first, soil from chrysanthemum cultivation, naturally infected with nematodes of the *Pratylenchus* spp. genus, was distributed into experimental units consisting of plastic 6 × 9 bags, each containing 400 g of soil. After determining the initial number of nematodes by the sieve-facial tissue paper method, the bags were divided into groups of 10 that received the different experimental treatments: the raw filtrate at 50, 70, 90 and 100 %, distilled water, a chemical nematicide (RUGBY®) and PDB Oxoid™ medium at 1 % [9]. Then, the decrease in nematode counts per 100 g of soil was assessed by the sieve-facial tissue paper method at 24 and 48 h post-treatment.

In the second experiment, sterile soil was packed into bags as in the first experiment, inoculated with nematodes of the Meloidogyne spp. genus (2,500 eggs per bag), divided into experimental groups of 10 units each and subjected right away to the same treatments employed in the first experiment. Then, tomato seedlings were planted on the treated samples (one per experimental unit) at 24 h post-treatment, and subjected again to the same experimental treatments 15 days later. Three months afterwards, the gall index on the resulting tomato plants was determined using the Taylor Sasser (1978) scale [11], as well as the number of nematodes and of juveniles stage 2 (j2) per 100 g of soil.

In the third experiment, sterile soil was packed into bags, inoculated with nematodes, split into experimental groups and subjected to the same treatments as in the second experiment. However, in this occasion the bags were left for three different time periods, T1 (3 days), T2 (6 days) and T3 (9 days) before planting tomato seedlings in them. Three months afterward, the same variables assessed in experiment 2 were evaluated.

Stage 2: Evaluation of the bioformulate

The *Purpureocillium* sp. strain UdeA0106 bioformulate was subjected to quality control testing (purity and germination percentage) before evaluation on the field. In all cases, the bioformulate complied with all applicable quality requirements pursuant to standing Colombian regulations [8].

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Nematode control under field conditions

Performance evaluation of the UdeA0106 bioformulate under production conditions spanned a period from year 2015 to year 2016, at the commercial chrysanthemum cultivation facilities of the Miramonte 2 ranch in the Marinilla municipality (Antioquia-Colombia). The experiment took place at each of two independent 36 m^2 beds, selected because they were naturally infected with plant-parasitic nematodes and did not exhibit detectable *Purpureocillium* spp. populations, according to initial sampling. With the purpose of minimizing the effect of confounding variables on the assessment of experimental treatments, the beds were held in a month-long quarantine before starting the evaluation, during which time they were not planted, fertilized or subjected to any type of commercial floricultural operation by ranch personnel.

Experimental design

The beds were divided structurally into 30 quadrants 1.17 m^2 each (Figure 1), which constituted the experimental units subjected to the different treatments. Each quadrant was randomly assigned to one of six specific treatments (Table 1); there were five replicates for each treatment on each bed, and the experiment as a whole was repeated twice independently. Owing to temporary supply issues, the experiment used two varieties of nematode-susceptible chrysanthemums (VERO and FACTOR).

The treatments were applied by drenching each quadrant with 5 L of the relevant suspension at 10^9 con/mL. These applications were performed 48 h before planting the beds and once every 8 days afterward until week 7, totaling 8 applications/bed. The chemical nematicide Furadan® was applied only twice; the first time before planting the seedling and the second, during week 7.

Performance variables were evaluated at the end of the production cycle (week 11). They were: the number of juvenile nematodes per 100 g of soil (evaluated in 5 random spots per quadrant, using the sieve-facial tissue paper method [8]), the number of galls, and the gall index in roots according to Taylor *et al*. [11].

Effect of the bioformulate on production parameters

The effect of the application of the bioformulate on production parameters was assessed by randomly taking 10 plants from each of five quadrants of the same treatment, and measuring weight, height, stem diameter and the number of flower buds per plant.

Statistical analysis

Statistical analysis of the data from the evaluation of both the raw filtrate and the bioformulate was performed using ANOVA with 0.05 as cut off for statistical significance in order to detect the presence of significant inter-treatment differences, and using multiple comparison tests to identify significantly different treatments. Data not fitting a normal distribution were analyzed using non-parametric Kruskal-Wallis tests and a Kruskal multiple comparison (Kruskalmc test). Initially the data of each time point were analyzed individually, but once it was statistically verified that time did not influence the result, they were all pooled and analyzed together. Statistical tests were performed as implemented in the R statistical software application [12], specifically, in the 'pgirmess' R package.

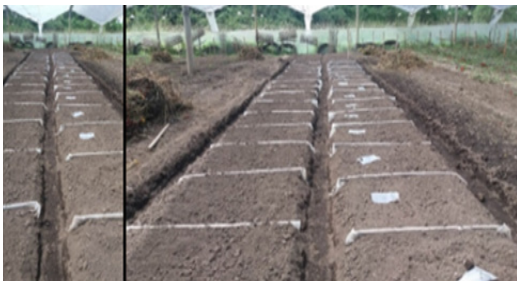

Figure 1. Partition in quadrants of each bed (experimental units) before field experiments with *Purpureocillium* sp. strain Udea0106 against *Meloidogyne* spp. and *Paratylenchus* spp. in Chrysanthemum.

Table 1. List of treatments used in field experiments to evaluate the effect of *Purpureocillium* sp. strain Udea0106 against *Meloidogyne* spp. and *Paratylenchus* spp. in Chrysanthemum

Treatment	Composition	Description
T1	*Purpureocillium lilacinum*	Strain obtained from the Miramonte SA collection, isolated from *Meoloidogyne* spp. juveniles and grown on rice
T2	RIZOCINUS®	*Purpureocillium lilacinum*-based commercial bioformulate, part of the product catalog of Ecosphaira®
T3	UdeA0106	Strain supplied by the FITOBIOL group of the University of Antioquia and formulated by Ecosphaira®
T4	Excipient	Excipient used to formulate strain UdeA0106 in RIZOCINUS®. Exact composition not disclosed owing to confidentiality agreement
T5	Chemical nematicide (Furadan®)	Carbamate pesticide with activity mainly against soil nematodes and insects, as well as some insects attacking plant leaves. A granulated presentation that dissolves easily in water was used in this work
T6	Negative control	Water applied in the same volume and periodicity as the other treatments

Results

Stage I: Evaluation of the filtrate

Nematode control in soil

The first experiment evaluated the effect of a 24 h-incubation of soil naturally infested with plant-parasitic nematodes of the *Paratylenchus* spp. genus with raw filtrates of the UdeA0106 strain. The percentage decrease on nematode populations was significantly higher for the 100 % and 90 % filtrates, which produced a drop in the number of j2 per 100 g of soil close to 100 % (Figure 2).

When the treatments are compared with the controls, there is a noticeable increase in the number of j2 in the H_2O and PDB controls, where no statistically significant differences were found between the filtrate concentrations, but it was possible to detect a difference between the PDB control and the 50 and 100 % concentrations of the filtrate (Figure 2).

When analyzing data on the number of juvenile-stage individuals of the *Paratylenchus* spp. genus left after a 48 h incubation with raw filtrates of *Purpureocillium* sp. strain Ude0106, compared to the number of nematodes before applying the experimental treatments, a decrease percentage higher than 80 % was

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found for filtrate concentrations of 90 and 100 %, and a decrease percentage higher than 40 % in the other analyzed filtrate concentrations (50 and 70 %), compared to the controls. In the controls, nematode populations actually increased, as reflected by their negative decrease percentages (–10 %). Statistically significant differences were found for the nematicidal effect of the higher analyzed filtrate concentrations as compared to the controls, after 48 h (Figure 2).

Nematode control in planta

After two applications (day 0 and day 15) of the different raw filtrate concentrations of strain UdeA0106 of *Purpureocillium* sp., it was found that the higher the filtrate concentration, the lower the number of *Meloidogyne* spp. j2 (Figure 3). There were statistically significant differences between the controls (H_2O and PDB) and the highest concentrations of the filtrate (90 and 100 %), where there were less than 20 juveniles per 100 g of soil.

When the effect of raw UdeA0106 on the appearance of root-knot galls was evaluated (Figure 4), it was found that the higher the concentration of the filtrate, the lower the number of galls per plant. The highest filtrate concentration (100 %) produced a 2.4-fold decrease on the gall index compared to the controls, where this variable reached the highest value (5) of the scale [11].

Preventive effect of the raw filtrate

The evaluation of the preventive effect of the raw filtrate revealed statistically significant differences between the different filtrate concentrations and the controls at all examined time points. There was a trend towards a more pronounced nematicidal effect (that is, lower numbers of juvenile nematodes) with higher filtrate concentrations. However, we were unable to detect statistically significant differences between different treatment times before planting the seedlings, suggesting that the decrease in the number of *Meloidogyne* sp. juveniles is not related to the time lapse they spend in contact with the filtrate.

Based on the data regarding the number of root-knot galls per plant, the highest gall index (5) was exhibited by the controls (PDB and H_2O) and the treatment with raw filtrates from *Purpureocillium* sp. strain UdeA0106 at 50 % concentration, after pre-incubation of seedlings for either 3, 6 or 9 days before planting. Notably, when the filtrate was used at 70 %, the gall index was reduced to 3, and applying the filtrate at 100 and 90 % further reduced this paramater to 2 was achieved at the three evaluated time points.

Stage 2: Evaluation of the bioformulate

Nematode control under field conditions and the effect on root-knot galls in chrysanthemums

After evaluating the gall indexes, it was found that the treatments containing *Purpureocillium* spp. strains (*P. lilacinum*, Rizocinus® and UdeA0106) yielded lower numbers of root-knot galls and hence smaller gall indexes compared to the control (Table 2). The smallest average number of root-knot galls and gall index were produced by strain UdeA0106 (2 and 6.54, respectively). Although it did not exhibit statistically significant differences compared to the Rizocinus® commercial product, in the same manner, it was found that the chemical nematicide obtained the same gall index as the control and the Carrier (Figure 5).

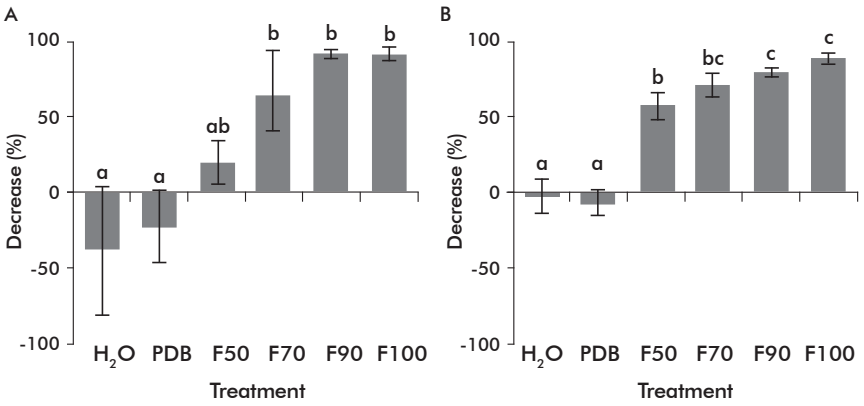

Figure 2. Effect on Juvenile stage *Paratylenchus* sp. nematodes of incubations with raw filtrates of *Purpureocillium* sp. strain UdeA0106. A) Effect after 24 h. B) Effect after 48 h. Different letters denote statistically significant differences according to Tukey's test ($p \leq 0.05$). F100-F50 stand for the filtrate's dilution percentages. PDB: PDB Oxoid™ medium at 1 %. Error bars stand for standard deviation.

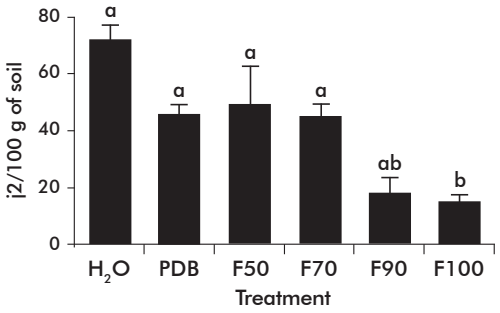

Figure 3. Number of juvenile stage *Meloidogyne incognita-javanica* nematodes found in the soil of tomato plants three months after the application of raw filtrates of *Purpureocillium* sp. strain UdeA0106 at different concentrations. Different letters denote the presence of statistically significant differences according to Tukey's test ($p = 0.05$). F100-F50 stand for the filtrate's dilution percentages. PDB: PDB Oxoid™ medium at 1 %. Error bars stand for standard deviation.

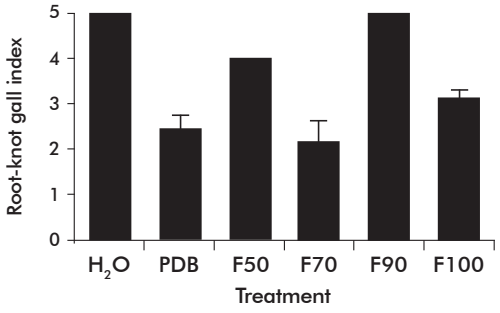

Figure 4. Effect of the application of raw filtrates from *Purpureocillium* sp. strain UdeA0106 on gall index A (according to Taylor and Sasser, 1983) in tomato plants. F100-F50 stand for the filtrate's dilution percentages. PDB: PDB Oxoid™ medium at 1 %. Error bars stand for 1 SD of the mean.

Evaluation of the bioformulate on Meloidogyne spp. juveniles in soils of chrysanthemum crops

During initial testing, *Meloidogyne* spp. was the most frequently isolated plant-parasitic nematode among all experimental units. This result is consistent with published research for chrysanthemum and other crops and is the reason this species is held as the most important plant-parasitic nematode from an economic point of view [11,13,14].

The control exerted by the treatments examined here on juvenile individuals of *Meloidogyne* spp. in chrysanthemum soils was evidenced mainly in those containing strains of the *Purpureocillium* spp. fungus, as they exhibited the lowest number of infectious-stage nematodes per 100 g of soil and at the same time, statistically significant differences with the control (Figure 6). The lowest number of juveniles was exhibited by experimental units that received the UdeA0106 formulation, finding an average lower than 400 *Meloidogyne* spp. juveniles per 100 g of soil, representing a higher than 50 % decrease compared to the control (Figure 6).

Evaluation of the treatments on Paratylenchus spp. juveniles in soil of chrysanthemum crops

The control treatment with the chemical nematicide (Furadan®), of low efficacy against *Paratylenchus* spp., similar to its effect on *Meloidogyne* spp. juveniles, was statistically indistinguishable from the control (Figure 7). This mirrors the behavior previously observed against *Meloidogyne* spp. juveniles and suggests the emergence of resistance against this nematicide among the examined populations of this nematode. The two treatments that most efficiently reduced the number of juvenile nematodes were Rizocinus® and UdeA0106, both including *Purpureocillium* spp. as a biological control agent.

Evaluation of the effect of the bioformulate on production parameters

The present study failed to find statistically significant differences between the evaluated treatments and the control regarding plant height (Figure 8A), which was 75.3 cm on average. Likewise, there were no statistically significant differences regarding the number of flower buds per plant between the treatments and the control (Figure 8B). The mean number of flower buds per plant across all experimental units was 10. Another morphological parameter relevant for export markets is stem diameter, which is determined both by the photoperiod the plant is subjected to and nutrient absorption [15]. This study also failed to find statistically significant differences between the treatments and the control regarding stem diameter (Figure 8C). Mean stem diameter in all examined treatments was 5 mm.

Another important productivity parameter is the weight per plant, shown in Figure 8D across all evaluated treatments. This parameter exhibited statistically significant differences in the plants treated with strain UdeA0106 when compared to all other treatments and controls. Whereas the average weight per plant reported by other authors for chrysanthemum is 40 g [15], the plants treated with UdeA0106 had a mean weight of 41.4 g, evidencing an increase in weight of 4.6 g compared to the control (36.8 g).

Table 2. Mean number of galls per plant and gall indexes per treatment in chrysanthemum plants*

Treatment	Mean gall number/treatment	Gall index
Rizocinus®	11.36	3
P. lilacinum	10.74	3
UdeA0106	6.54	2
Carrier	13.93	4
Furadan®	31.02	4
Control	32.19	4

* Rizocinus®, *P. lilacinum* and UdeA0106 strain treatments contain *Purpureocillium* spp. strains.

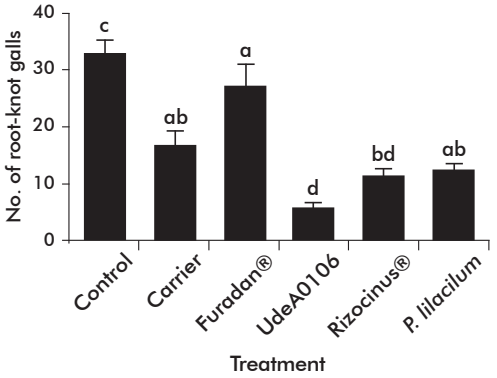

Figure 5. Mean number of root-knot galls per chrysanthemum plants after treatment with *Purpureocillium* sp. or controls. The bars represent the standard error of the mean according to the Kruskal-Wallis test. Different letters denote statistically significant differences between treatments according to the kruskalmc multiple comparison test. UdeA0106: *Purpureocillium* sp. strain Udea0106.

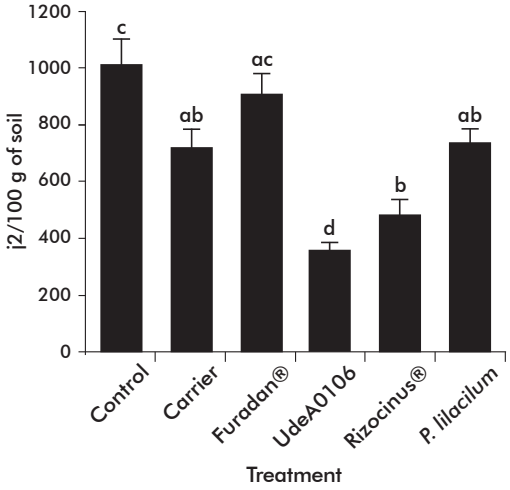

Figure 6. Effect of the treatments on juvenile-stage *Meloidogyne* spp. nematodes in chrysanthemum soils (mean number of j2 per 100 g of soil). Values are presented as means plus the standard error of the mean. Different letters denote statistically significant differences between treatments. The analysis was performed with the non-parametric Kruskal-Wallis test and the kruskalmc multiple comparison test. UdeA0106: *Purpureocillium* sp. strain Udea0106.

Discussion

The biological control exerted by the filtrate and the bioformulate of strain UdeA0106 of *Purpureocillium* sp. on nematodes of the *Meloidogyne* spp. genus is similar to that observed by Gaviria in 2000 [16]. He noticed that *P. lilacinum*, when used as a control agent against juvenile stages of the *Meolidogyne* hapla-javanica complex in chrysanthemum beds from different farms of western Antioquia, had a higher nematicidal efficiency than compost and a reference chemical pesticide [16]. Purpureocillium sp. has been previously shown to be highly pathogenic against different life cycle stages of *Meloidogyne* spp. [17-19], including eggs and the infective j2 stage [11], and to reduce, as a consequence, the number of root knot galls produced by this nematode genus [89-23]. It is worth noting that Victor & Fereres in 2005 [22] found that biological control agents such as *Purpureocillium* spp. tend to provide a more stable and long-lasting control than other alternatives owing to their ability to replicate in their target environments, and although the simultaneous inoculation of different biological control agents carries the potential risk of biological antagonism if not planned carefully, the treatments studied in the present work were applied separately to avoid possible interferences between potential combinations.

The low efficiency of Furadan® in the control of root-knot galling produced by nematodes observed during the present work has been previously noted by other researchers [20], who found that Furadan® failed to decrease the size of nematode populations in soil and the number of root-knot galls in different crops to the extent achievable with *Purpureocillium* spp. strains or similar products [25, 20].

The lower number of root-knot galls observed upon treatment with the conidia and raw filtrate present in the bioformulation of strain UdeA0106 has two possible mechanisms of action: first, a direct effect of this bioformulate on infective nematode stages in soil [24] and second, interference with the attack of nematodes and other phytopathogens by the fungus acting as a root endophyte, as has been previously described in the literature for *Purpureocillium* sp. [27, 28].

It should be noted that despite the appearance of recent publications describing *Purpureocillium* sp. as an opportunistic human pathogen, the application of this bioformulate to chrysanthemum cultivation does not pose a human health hazard when the final destination of this product is the foreign cut flower market, as the bioformulate is applied solely by drenching the soil during cultivation by personnel who has been properly trained on all pertinent biosafety procedures and therefore does not contact directly aerial plant structures. Additionally, in the cut flower market the stems are packed without roots or soil [27] and hence, there would be no traces of the UdeA0106 bioformulate in the final product.

Also, the present work, which describes the effective control of *Paratylenchus* spp. by raw filtrates and a bioformulate of strain UdeA0106, joins the still small number of publications reporting that this nematode can be controlled by *Purpureocillium* sp. In 1999, for instance, it was found that *P. lilacinum* decreased the number of juveniles of this species compared to the

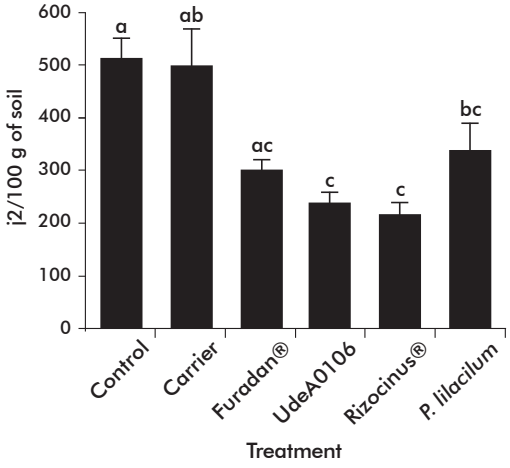

Figure 7. Effect of the treatments on juvenile-stage *Paratylenchus* spp. nematodes in chrysanthemum soils (mean number of j2 per 100 g of soil). Values are presented as means plus the standard error of the mean. Different letters denote statistically significant differences between treatments. The analysis was performed with the non-parametric Kruskal-Wallis test and the kruskalmc multiple comparison test. UdeA0106: *Purpureocillium* sp. strain Udea0106.

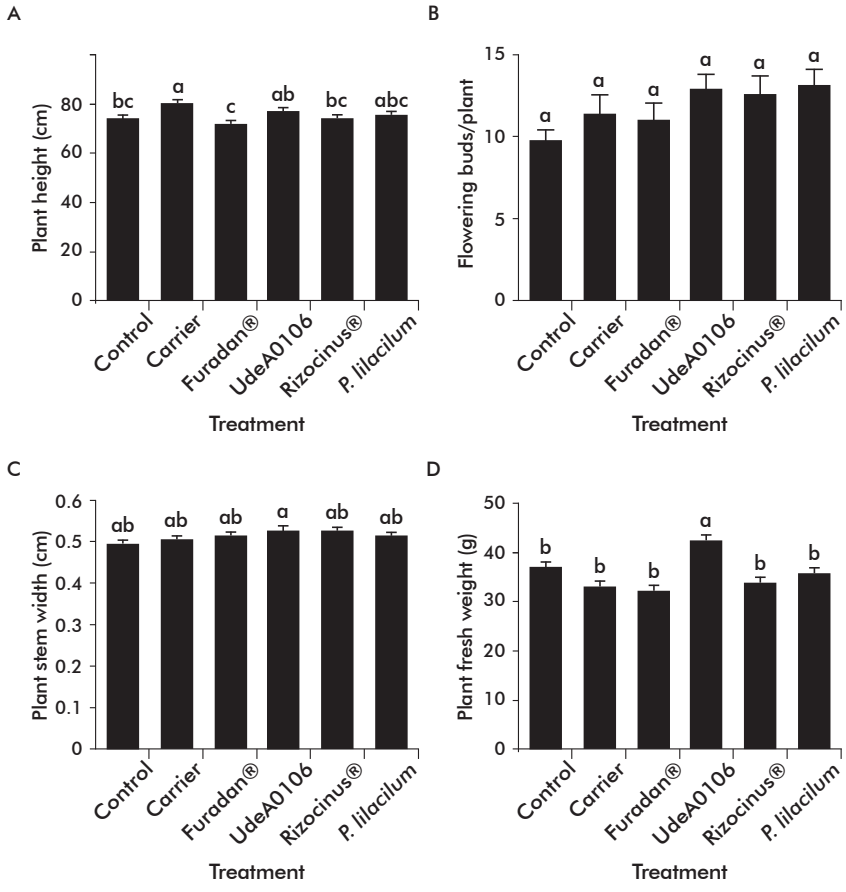

Figure 8. Effect of the bioformulate of *Purpureocillium* sp. strain UdeA0106 on production parameters of chrysantemum plants. A) Plant height. B) Flowering buds. C) Stem width. D) Fresh weight. The analysis was performed with the non-parametric Kruskal-Wallis test and the kruskalmc multiple comparison test. UdeA0106: *Purpureocillium* sp. strain Udea0106.

control [29]. This is an important finding, as in 1982 *Paratylenchus* spp. was already reported by Meredith *et al*. [28] as the most relevant plant-parasitic nematode for the cultivation of chrysanthemums in Venezuela.

A standardized set of governmental norms and regulations addressing the quality requirements that chrysanthemum growers must meet when producing cut flowers destined to foreign markets does not exist [27], owing perhaps to the fact that such requirements would depend not only on flower species, but on the requirements of the importing country. Among the most important quality parameters are flower color, variety, shelf life, height, stem width, stem length and weight [32, 33].

The attack of nematodes to susceptible varieties results in damage to the radicular system and consequently, reductions in the weight and height of the plant, among others [32]. Although the present study failed to find an effect of nematode infestation on these parameters, such a finding is not unheard of in published research [35, 36].

The application of strain UdeA0106, however, did produce an increase in stem width, which is explained by the reduction it produced in the number of juvenile stage nematodes for the two studied genera and hence, better root health, nutrient absorption and larger plant weights. There is indeed a direct relationship between plant weight and nutrient absorption, as described by Gaytán *et al*. in 2006 [30]. Another study, published by Ocampo *et al*. [35], found differences between the fresh and dry weights of healthy plants and plants infected with *Paratylenchus* spp.

On the other hand, previous studies have obtained results similar to those shown here regarding the promotion of plant growth upon treatment with *Purpureocillium* sp. strains. Hajji *et al*., for instance, detected that the application of *P. lilacinum* increased weight by 64 and 94 % in potato plants attacked by *Meloidogyne* spp. and *Globodera* spp., respectively [36], and other authors have shown improvements in plant growth in chickpea when the *M. incognita* nematode population is controlled with this fungus [37]. A similar result was obtained in eggplant, where *P. lilacinum* was effectively used for the control of *Meloidogyne* spp. juveniles, yielding a decrease in the number of root-knot galls and an improvement on production parameters [38].

In summary, our study supports the application of raw filtrates and a bioformulate of *Purpureocillium* sp. strain UdeA0106 for the effective control of *Paratylenchus* spp. and *Meloidogyne* spp. nematodes in chrysanthemum (*Dendranthema grandiflora*) crops.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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