Bionatura Journal
Ibero-American Journal of Biotechnology and Life Sciences

Article

Biological nematicides as an alternative for control of *Meloidogyne incognita* populations in yellow pitahaya (S*selenicereus megalanthus*).

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Available from. http://dx.doi.org/10.21931/BJ/2024.01.01.4

ABSTRACT

Yellow pitahaya in the Ecuadorian Amazon has become one of the most important economic crops in the region. However, pests (nematodes) in the soil have affected up to 100% of the crop's growth stages. Faced with this problem, growers use various chemical nematicides that minimize this impact but cause contamination problems. For this reason, the objective of the research was to evaluate microorganisms that control or reduce the population of *Meloidogyne incognita* in the pitahaya crop at the greenhouse level. The design was DBCA, and the statistical analysis was performed with the statistical package Infostat 2017, using linear mixed models and Fisher's tests at 5%. The results show that root nodules decrease when P. lilacinum + T. asperellum is applied after nematode injection (261). In addition, the lowest number of nodulations (251) was obtained when microorganisms were applied after nematode inoculation (251 nodulations). Still, when microorganisms were used before, aerial biomass growth was stimulated (384.17 g) even when nematodes were present in the root system.

Keywords: microorganisms; nematodes; pitahaya.

INTRODUCTION

The pitahaya (*Selenicereus megalanthus* Haw.) is an exotic fruit cultivated in various Latin American countries such as Mexico, Central America, Venezuela, Ecuador, Colombia, and Peru [1]. Recently, countries such as Panama, Uruguay, Thailand, and Indonesia have also started cultivating this fruit due to its functional properties [2].

In Ecuador, it is estimated that there are around 2000 hectares dedicated to pitahaya cultivation, mainly in the provinces of Pichincha, Manabí, and in the Amazon region in Morona Santiago, Orellana, and Sucumbíos [3]. In the Amazon region, pitahaya cultivation is commercially a monoculture using conventional agronomic methods. This is mainly due to the vulnerability of pitahaya to various pests and diseases, such as fungi, bacteria, viruses, insects, and nematodes, which affect the plantations at all stages of growth. The most

common nematodes affecting pitahaya belong to the genera *Meloidogyne* sp. (50-81%), *Helicotylenchus dihystera* (82-100%), *Hemicycliophora* sp., *Tylenchorhynchus* sp., *Xiphinema* sp., *Trichodorus* sp., *Hoplotylus* sp., *Hemycicliophora* sp., *Dorylaimus* (27%), *Tylenchus* (23%), *Aphelenchus* (14%), and *Pratylenchus* (5%) [4-5]. In the Palora canton, which hosts the largest cultivated area of pitahaya, it has been observed that 97% of the plantations are affected by *Meloidogyne* sp. and *Helicotylenchus* spp.. In comparison, 3% are impacted by *Tylenchus* ssp. [6]. Nematode infestation, especially of the genus *Meloidogyne* sp., in the root system of pitahaya plants causes a decrease in crop yield. This is due to the formation of nodules in the roots, which hinders water absorption and nutrients from the soil [7-8]. Additionally, visible symptoms in the aboveground part of the plants include yellowing, thin and weak stems [9,6,5].

Currently, non-fumigant products, such as organophosphates and carbamates, chemical compounds with nematicidal activity, are used for nematode control in pitahaya cultivation. However, these products present environmental risks and can be toxic to humans [10]. Studies conducted in different crops, such as lettuce, tomato, cauliflower, celery, and broccoli, have found pesticide residues (organophosphates and carbamates) in 48% of the analyzed products [11-13]. Furthermore, it has been observed that 3% of agricultural workers exposed to pesticides suffer annually from chronic intoxication, neurological disorders, peripheral neuritis, male hormonal alterations, optic nerve problems, cataract formation, and respiratory effects [12].

Given that nematode control in pitahaya crops is mainly carried out with highly toxic nematicides, it is urgent to seek alternatives to the use of pesticides. In this regard, biological control through antagonistic organisms has been the research subject in recent years, and its potential for managing plant-parasitic nematodes has been recognized [14]. These beneficial organisms include *Pasteuria penetrans, Pasteuria hartismeri, Pochonia chlamydosporia, Bacillus firmus, Paecillomyces lilacinus,* and *Trichoderma* spp. These microorganisms act by adhering to the nematodes' cuticle or parasitizing the females' eggs, resulting in the death of the nematodes. Although variable results have been obtained in research on antagonistic organisms, their effectiveness in controlling plant-parasitic nematodes has been demonstrated. However, only a tiny group of antagonistic organisms has been studied in detail [15].
For this reason, this research aimed to evaluate microorganisms that control or reduce the population of Meloidogyne incognita in the pitahaya crop at the greenhouse level. For this study, strains of microorganisms from pitahaya plantations, which have been selected at the in vitro level, were used. In addition, a commercial product based on *P. lilacinum* + *T. asperellum* was employed because there are products that no longer have viable fungal spores when they do not receive a good storage process.

MATERIALS AND METHODS

Location of the Experiment

This study was conducted at the Central Experimental Station of the Amazon (EECA), National Institute of Agricultural Research (INIAP), in Orellana, La Joya de los Sachas canton province. The experimental site was situated at 0291649 latitude and 09962311 longitude, with an altitude of 282 meters above sea level. The climate in the area is warm and humid tropical, with an average annual temperature of 25°C, an average maximum temperature of 22°C, an average minimum temperature of 40°C, and an average relative humidity of 90%. In the greenhouse, the average relative humidity is 70%, and the average temperature is 35°C

Treatments

The experiment was organized in a randomized complete block design with three replications. The experimental unit consisted of eight pots with yellow pitahaya cuttings. The treatments consisted of T1 (*Purpureocillium lilacinum*) Laboratory strains, T2 (*Trichoderma asperellum*) Laboratory strains, T3 (*Purpureocillium lilacinum* + *Trichoderma asperellum*) Laboratory strains, T4 (*Purpureocillium lilacinum* + *Trichoderma asperellum*) Comercial product, T5 (absolute control), and T6 (control + nematode). The microorganisms stored in the laboratory and used for this study already come from a previous research process; they arrive from pitahaya plantations.

Specific Management of the Experiment

The study was implemented under greenhouse conditions. Yellow pitahaya cuttings of 40 cm were planted in pots with 4500 g of sterilized soil at 2 to 3 cm depth. After 22 days, the cuttings began to emit their first roots [16].

M. incognita was extracted from the galled roots of yellow pitahaya. The roots were washed, cut into approximately 1 cm sections, and blended with 100 ml of water in a blender for 20 seconds in two intervals with five seconds of rest. Subsequently, the blender content was passed through a set of nested sieves with 250, 150, and 25 µm openings (mesh sizes of 60, 100, and 500, respectively). The content placed on the 250 and 150 µm sieve was rinsed with running water for 1 minute. The sediment on the No. 500 sieve was collected in a graduated cylinder and filled with 100 ml of water. It was then homogenized with an air pump, and a 4 ml aliquot was taken for nematode identification and counting using a trinocular inverted microscope with LWD IOS objectives, X-LED illumination, and EWF10X/22mm eyepieces [17].

Finally, *M. incognita* was inoculated into 15-day-old tomato plants (*Lycopersicum esculentum* Mill) from transplant. After 30 days, nematodes were extracted from the *L. esculentum* Mill plants and inoculated into pitahaya plants. Approximately 1200 J2 nematodes were applied. Four 5 cm deep holes were made in the soil near the base of the plant stem for injection, the nematode solution was poured, and then the holes were covered [18,19].

The biological control agents used were *T. asperellum* and *P. lilacinum*. Spore crystals were taken with sterile forceps and seeded on Petri dishes containing Potato Dextrose Agar (PDA) medium [20]. Sterilized rice substrates were used to mass produce the control agents and a conidial suspension of *T. asperellum* and *P. lilacinum*. The conidial suspension was prepared by adding 20 ml of sterile distilled water with 0.1% Tween 80 to each Petri dish containing *T. asperellum* and *P. lilacinum* with 6 days of growth. To this conidial suspension, 125 ppm of chloramphenicol was added. Subsequently, 3 ml of each suspension was taken using a sterile pipette and deposited in bags containing 150 g of the sterilized substrate (rice), which were then incubated for 15 days at a room temperature of 24 ± 2 °C [21]. To establish the inoculum concentration, 1 g of rice from each multiplied substrate was weighed, and a suspension was prepared in 10 ml of distilled water. The spore quantification was performed by performing 20 readings in a Neubauer chamber [22]. Once the concentration was determined, $1x10^{-9}$ spores were applied in 100 ml solutions per plant 7 days before and 7 days after the injection of M. incognita.

Evaluation Methods

At 30, 60, and 90 days after injection of *M. incognita*, a destructive evaluation was performed on 2 plants (treatment and replication). These plants were randomly selected, and the following parameters were assessed: incidence, severity, aboveground biomass weight, and final nematode population (PF).

a) **b)**

Figure 1. Nematode Sampling in *S. megalanthus* Plants. a) Extraction of the plant and sampling .b) Register agronomic variables (plant and roots).

The number of plants infected by M. incognita was recorded 30, 60 and 90 days after inoculation with the nematode to determine the incidence. The results were expressed as a percentage (%) [23]. To select the galling index (GI) in the root system of pitahaya plants, the number of galls formed in the root was counted and with the scale proposed by Taylor and Sasser [28] (0 to 6, where 0 = 0 galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; 5 = >100 galls) the severity was estimated [24].

All cladodes were collected per plant to determine aerial fresh weight (AFW). An analytical balance was initially used to define the fresh weight in grams (g). These procedures were carried out according to the study conducted by Gelpud et al. [25]. The multiplication rate of nematodes in soil and roots (MR) was also determined by dividing the final population (Pf) by the initial population (Pi) Berroterán et al. [26].

Statistical analysis was performed using the statistical package Infostat version 2017, and variance analysis was conducted using generalized linear mixed models. The difference between the means of the treatments was estimated using the Least Significance Differences (LSD) Fisher with a significance level of 5% [27].

RESULTS

For the nematode incidence variable, it was determined that all plants in the different treatments were affected by nematode infestation (ranging from 89% to 100%) (Table 1), demonstrating that the applied inoculum concentration (1200 J2) caused an infection process. The presence of nematodes in the absolute control may be attributed to potential contamination of the experimental units during irrigation.

Treatment	Number of affected plants (before)	Incidence (%)	Number of affected plants (after)	Incidence (%)
*P. lilacinum**	8	89	16	89
*T. asperellum**	9	100	18	100
P. lilacinum + *T. asperellum**	9	100	18	100
P. lilacinum + *T. asperellum***	8	89	17	94
Absolute control	4	44	4	22
Control + nematodes	6	67	18	100

* Laboratory strains; ** Comercial product.

Table 1. Incidence of M. incognita in H. megalanthus plants.

A univariate analysis was performed for the variable "number of nodules" (Table 2). A highly significant difference was found for treatments ($p<0.0001$), a significant difference for the time of application "before and after," and the interaction (days*treatment) ($p=0.0026$; $p=0.0170$ respectively).

The main effect for treatments indicated that the lowest number of galls was formed when *T. asperellum* was applied. However, it was observed that the combination of the commercial product *P. lilacinum* + *T. asperellum* ($1x\ 10^{-11}$ cfu/g) resulted in a lower number of galls compared to the control + nematode. The treatments where laboratory-obtained strains were applied (*P. lilacinum* + *T. asperellum* and *P. lilacinum*) at concentrations of $1x10^{-9}$ spores yielded the highest number of galls (Table 3). According to the scale reported by Taylor and Sasser [28], the severity grades for the different treatments were 5, meaning that the number of galls in the experimental units was high (more than 100 galls) (Table 3).

Treatment	Number of gills
Treatment	**
Times of application	*
Times of application * treatment	*

** significant at $p \leq 0.01$, * significant at $p \leq 0.05$.

Table 2. The main effects and interaction effect for the number of galls on roots determined for each factor: Treatment and Time of application.

Treatments	Number of gills
P. lilacinum + *T. asperellum**	481 a
*P. lilacinum**	438 a
Control + nematodes	423 ab
P. lilacinum + *T. asperellum***	261 bc
*T. asperellum**	248 c
Absolute control	101 c

* Laboratory strains; ** Comercial product.

Table 3. Mean values of the number of galls in the root system of *H. megalathus.*

The analysis of the interaction treatment * season of application shows that the commercial product based on *P. lilacinum* + *T. asperellum* presents the least formation of nodulations at 60 and 90 days of evaluation. With *T. asperellum* the opposite happened, the lowest number of nodules was found at 30 days and as time passed the number of nodules increased (Table 4). On the other hand, the best time of application of the treatments was when the controllers were applied after the inoculation of the nematodes (251 nodulations) with respect to the 400 nodulations obtained when it was applied before (Table 4).

Treatments	Days	Number of gills
P. lilacinum + *T. asperellum**	30	522 abc
*P. lilacinum**		518 abc
P. lilacinum + *T. asperellum***		387 bcde
Control + nematodes		341 bcdef
*T. asperellum**		218 def
Absolute control		69 f
Control + nematodes	60	677 a
*P. lilacinum**		494 abcd
P. lilacinum + *T. asperellum**		377 bcde
*T. asperellum**		246 cdef
P. lilacinum + *T. asperellum***		214 def
Absolute control		122 ef
P. lilacinum + *T. asperellum**	90	542 ab
*P. lilacinum**		301 bcdef
*T. asperellum**		279 bcdef
Control + nematodes		251 cdef
P. lilacinum + *T. asperellum***		180 ef
Absolute control		111 ef

* Laboratory strains; ** Comercial product.

Table 4. Mean values of the number of galls by treatment and season of application.

The analysis in the air fresh weight (PFA) variable showed highly significant differences for the application time factor and treatments ($p<0.0001$) and not significant for the interaction ($p=0.4632$) (Table 5).

Treatment	Aerial biomass (g)
Time of application	**
Treatment	**
Time of application * treatment	NS

** significant at p ≤ 0.01; * significant at p ≤ 0.05; NS not significant.

Table 5. Main effects and interaction effect for aerial biomass determined for each factor: Treatment and Time of application.

It was determined that the amount of aerial biomass was higher when the biological controls were applied before the injection of nematodes (384.17 g) than when applied after (314.74 g). The main effect of the treatments showed that the amount of aerial biomass was higher when *P. lilacinu*m + *T. asperellum* (obtained at laboratory level) was applied. On the other hand, it was observed that when *T. asperellum* obtained at the laboratory level was involved, the amount of aerial biomass was the lowest (Table 6).

Treatments	Aerial biomass (g)
Absolute control	422.02 a
P. lilacinum + *T. asperellum**	365.40 b
*P. lilacinum**	352.17 bc
Control + nematodes	330.61 bc
P. lilacinum + *T. asperellum***	316,13 c
*T. asperellum**	310,40 c

* Laboratory strains; ** Comercial product.

Table 6. Aboveground biomass (g) of *H. megalanthus*.

The analysis of the variable number of nematodes in the soil showed significant differences for the application times factor (p=0.0011) and non-significant differences for treatments and interaction (p=0.6139, p=0.2152, respectively). (Table 7).

Treatments	Nematodes number
Time of application	*
Treatments	NS
Time of application x treatments	NS

* significant at p ≤ 0.05, NS not significant.

Table 7. Main effects and interaction effect for the number of nematodes in soil determined for each factor:

Treatment and time of application.

The lowest amount of nematodes in the soil was found when the inoculation with the biocontrol agents was carried out before the inoculation of *M. incognita* (Table 8). However, this did not help to control the damage suffered by the roots of the pitahaya plants.

Time of application	Soil nematodes number
Before	8857 b
After	22636 a

Table 8. Population of nematodes in the soil in two seasons of applications (before and after).

When analyzing the variable number of nematodes in the roots system of the pitahaya plants, significant differences were found for the time of application of the biocontrols (p=0.0198) and non-significant differences for the treatments and the interaction (p =0.5402, p=0.1429, respectively) (Table 9). That is, the biological controls, when applied before, did positively influence the population density of M. incognita (Table 10).

Treatments	Nematodes number
Time of application	*
Treatments	NS
Season x Treatments	NS

* significant at $p \leq 0.05$, NS not significant.

Table 9. The main effects and interaction effect for the number of nematodes in S. megalanthus root were determined for each factor: Treatment and time of application.

Time of application	Root nematodes number
Before	13199 b
After	21132 a

Table 10. Population of nematodes in the root system of S. megalanthus plants.

DISCUSSION

The number of galls in the root system of yellow pitahaya (*S. megalanthus*) indicates the plant's susceptibility to attack by *M. incognita*. In this study, the number of galls decreased by 62% and 70% when *T. asperellum* (laboratory strains) and *P. lilacinum* + *T. asperellum* (commercial) were applied, respectively. The excellent performance of *Asperellum* may be because it was collected in pitahaya plantations. It was also determined that applying *P. lilacinum* and *P. lilacinum* + *T. asperellum* strains obtained in the laboratory produced 4% and 13% more galls than the control + nematodes. This behavior could be attributed to certain micro-organisms possessing root growth-promoting properties, especially micro-organisms of the genus Trichoderma. This behavior could potentially be attributed to the root growth-promoting properties of the Trichoderma genus. This behavior is supported by Brotman et al. [29], who mentioned that Trichoderma activates auxins and several genes responsible for plant root development. However, Kariga et al. [30] mention that T. asperellum M2RT4 reduced the number of galls, egg mass, and nematode eggs when placed. On the other hand, when both

microorganisms are applied together, they seem to exert more efficient control, as *T. asperellum* T203 colonizes the roots and *P. lilacinum* reduces root nodulation, mass and egg generation while favoring host growth. [30].

At 30 days, the presence of *T. asperellum* minimized nematode attack on the pitahaya root system, possibly because the concentration decreased by 26% at 60 days [31]. At 60 and 90 days, it was observed that *P. lilacinum* + *T. asperellum* (commercial) resulted in lower nodule formation compared to the control + nematode and the laboratory-obtained strains of *T. asperellum* and *P. lilacinum*. This may be attributed to the stable germination of the microorganisms [31]. The lowest amount of galling was observed when the organisms were applied after nematode inoculation, which differed from the reported behavior of reducing root galling, egg mass, and egg production when the microorganisms were applied ten days before (reference not provided).

Applying the microorganisms seven days earlier positively influenced plant growth (aboveground biomass). This is possible because the microorganisms stimulate the growth of the root system. Kariga et al. [30] noted that applying *T. asperellum* M2RT4 and *P. lilacinum* (MR2 and KLF2) increased host growth and reduced nematode populations in soil and roots. Silva [32] also determined that *P. lilacinus* and several Trichoderma species promoted root development, growth and plant production when the microorganisms were applied before Meloidogyne inoculation. Also, it was resolved that plants had higher aerial biomass when inoculated with *P. lilacinum* + *T. asperellum* and laboratory-derived *T. asperellum*. This behavior may be attributed to these microorganisms being collected in pitahaya plantations in the Ecuadorian Amazon, which is why they performed better. Rodriguez-Kabana et al. [33] highlight the substantial divergence in the behavior of microorganisms, particularly concerning their efficacy in biocontrol and their proficiency in establishing themselves within the soil. This underscores the necessity of ensuring harmonious compatibility with distinct local conditions. For instance, Ortiz et al. [34] noted an illustrative case wherein the application of *P. lilacinum* on *Psidium guajava* to manage *Meloidogyne* spp. did not exhibit any detrimental impact on plant growth and development, even when nematodes were present within the root system. This finding is compelling in demonstrating that the behavior of microorganisms differs especially if they are used in other environments and are not native.

CONCLUSIONS

The application of the commercial dosage of *P. lilacinum* + *T. asperellum* led to a decrease in the number of galls present on the roots as compared to the untreated control. Nonetheless, no notable enhancement in plant growth was noted. Conversely, plant development was increased when employing a dosage of the laboratory-derived P. lilacinum + T. asperellum strain; however, the gall count did not decrease. These findings indicate that the strains under examination in this study possess constrained potential for nematode control.

This study established the importance of searching for new local strains and adjusting application rates since the behavior of microorganisms, especially in biocontrol and establishment capacity, can vary significantly from one place to another.

Author Contributions: Conceptualization, Y.V.-T., JP; methodology, Y.V.-T. and JP; statistical analysis, Y.V.-T., JP, writing—original draft preparation, Y.V.-T.; writing—review and editing, Y.V.-T., JP, NM, JS and AG All authors have read and agreed to the published version of the manuscript.

Funding:

This research was financed by the National Institute of Agricultural Research (INIAP) through the Central Experimental Station of the Amazon (EECA) and by the Research Fund for Agrobiodiversity, Seeds and Sustainable Agriculture (FIASA).

Data Availability Statement:

The data is contained in the article.

Acknowledgments:
This work was completed with the support of agronomists from the Fruit Programme of the Central Experimental Station of the Amazon.

Conflicts of Interest:

The authors declare no conflict of interest.

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Received: 9 October 2023/ **Accepte**d: 15 January 2024 / **Published:**15 February 2024

Citation. Vargas Y, Pico J, Manobanda N, Garcia A, Sanmiguel J.Biological nematicides as an alternative for control of *Meloidogyne incognita* populations in yellow pitahaya (S*selenicereus megalanthus*). Revis Bionatura 2024; 1 (1) 4. http://dx.doi.org/10.21931/BJ/2024.01.01.4

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Peer review information. Bionatura thanks anonymous reviewer(s) for their contribution to the peer review of this work using https://reviewerlocator.webofscience.com/