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Original article

**MULTIANTAGONISTIC OF *STREPTOMYCES*
NARBONENSIS STRAIN PSM242 AND *TRICHODERMA*
SP. BIOCIDES COMBINED IN ENRICH MEDIA
AGAINST *MELOIDOGYNE* SP. ROOT-KNOT
NEMATODE ON CHERRY TOMATO PLANTS**

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Abstract

The research focuses on the isolation and identification of *Streptomyces* and *Meloidogyne* species from soil and plant samples, particularly in oil palm plantations and tomato crops. Using the soil plating method, two *Streptomyces* isolates were obtained, identified as *Streptomyces narbonensis* through molecular techniques targeting the 16S rRNA gene. Concurrently, root-knot nematodes (*Meloidogyne* sp.) were extracted from symptomatic tomato plants using a modified Baerman apparatus, with morphological characteristics confirming their identity. The study further evaluates the biocontrol potential of *S. narbonensis* strain PSM242 and *Trichoderma* sp. against *Meloidogyne* sp. through both in vitro and field experiments. Results indicate that these biocontrol agents significantly reduce nematode populations and root gall formation, leading to enhanced growth metrics in cherry tomato plants. A factorial randomized block design was employed for data analysis, revealing that the combination of *S. narbonensis* and *Trichoderma* sp. in nutrient-enriched media yielded optimal results in controlling nematodes. Statistical analyses demonstrated that treatments combining both biocontrol agents resulted in the lowest gall diameter, weight, and juvenile nematode populations compared to controls. Additionally, significant improvements in root

length and fruit weight were observed in treated plants. This research underscores the potential of utilizing microbial antagonists as sustainable alternatives for managing agricultural pests, contributing to more effective pest control strategies in crop production systems.

Keywords: *Streptomyces*; *Trichoderma*; *Meloidogyne*; Biocontrol; Enrich Media

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Introduction

An essential type of plant-disturbing organism that often attacks tomato plantations is the root-knot nematode caused by the nematode *Meloidogyne* sp. [1]. This nematode causes swelling of the roots (root-knot). Losses caused by the nematodes *Meloidogyne* sp. can reduce tomato plant productivity by 20% to 40% [2]. Isolation and screening of *Streptomyces* sp. and *Trichoderma* sp. in tomato fields that often use pesticides found isolates that produce chitin, have antibiosis against fungi, and act as a bioinsecticide for several insects. Research [3] reported that the isolates of *T. harzianum* could control the root-knot nematode *M. javanica* by increasing plant growth, reducing the ability of nematodes to reproduce, and inhibiting the formation of galls on the roots of host plants. The results of a review of *T. harzianum* explained that this fungus is widely used as a biological agent for plant pests and diseases [4; 5]. The nematocidal activity of teleocidin B4 isolated from *S. narbonensis* can control the pinewood nematode, *Bursaphelenchus xylophilus* [6]. The results of an in vivo study revealed the potential of the nematocide *S. hydrogenans* to control root galls effectively, egg mass in tomato plants infested with nematodes, and at the same time promote the growth of tomato plants [7]. *S. narbonensis* produces Chitinase, which can degrade chitin in the cell walls of insect larvae *M. incognita* in vitro [8].

The combination of the biological agent *Trichoderma viridae* with *Pseudomomans florencens* increases the biocontrol effect on *M. javanica* activity compared to the administration of fungi and bacteria that stand alone [9]. The report shows that the 5:1 combination of *S. narbonensis* and *T. harzianum* on Potato Dextrose Extract media produces chitinase enzymes that can hydrolyze chitin cells [10; 11]. An important factor that affects the growth of biological agents is the nutrient content of the propagation or production

media [12; 13]. The type of propagation media will affect the level of ability of biological agents to control root-knot nematodes. Therefore, research was conducted on the potential ability of *T. harzianum* and *S. narbonensis* combine as a natural agent in the use of various culture mediums, namely EKG media (Potato Dextrose Extract), GN (Glucose nitrate), and coconut water (CW) in vitro.

Samples and research methods

Exploration of Streptomyces strains

Streptomyces isolates were obtained from soil collected around oil palm plantations using the soil plating method. A 1 g soil sample was weighed and prepared as a suspension with a 10^{-4} dilution. From this suspension, 1 mL was aseptically transferred selective media *Streptomyces* Isolation Medium Potato (SC) containing glucose, L-glutamic acid, K_2HPO_4 , $MgSO_4 \cdot 7H_2O$, NaCl, $FeSO_4 \cdot 7H_2O$, agar, and nystatin in a petri dish [14]. The isolated *Streptomyces* were then purified and cultured on SC medium in both petri dishes and test tubes for further propagation.

Molecular Identification of Streptomyces strains

Genomic DNA was extracted using the Presto™ Mini gDNA Bacterial Kit (Geneaid, USA) following standard protocols. Amplification of the 16S rRNA gene was performed using the primers 63F (5'-CAGGCCTAACA-CATGCAAGTC-3') and 1387R (5'-GGGCGGWTGTACAAGGC-3'). The PCR reaction mixture contained 1 µL of GoTaq® Green Ready Mix (Promega, USA), 0.5 µM of each primer, 10 µg of genomic DNA, and nuclease-free water, with a final volume of 50 µL. PCR products were resolved on a 1% agarose gel at 80 V for 45 minutes, and DNA bands were visualized using a UV transilluminator after EtBr staining. The amplified DNA was sent to a sequencing service provider for nucleotide sequencing. The sequencing data were analyzed using SeqTrace software and aligned with GenBank® database sequences using the BLASTn tool available on the NCBI website (<http://www.ncbi.nlm.nih.gov>).

Extraction of Meloidogyne sp.

Rhizosphere soil and root samples were explored from tomato plantations at the tomato production center in Pasuruan, Indonesia. Root sampling was carried out by purposive sampling on plants that experienced symptoms of root-knot nematode attack in the form of yellow wilting on the leaves and the formation of galls or root swelling [15]. Soil sampling was carried out in the root area of symptomatic plants of about 500 grams of soil/plant. Isolation of root-knot

nematodes by extraction-isolation method Modified Baerman apparatus. The identification method was a morphometric method to observe the morphology and characteristics of the nematodes.

Extraction The results of the exploration of root-knot nematodes were symptomatic roots and soil in the root area. Furthermore, in the extraction - isolation with a modified Baerman technique [16] for ± 24 hours. Then the nematode isolates were filtered using a nematode filter of 100, 250, and 500 mesh. The filtering results were observed using a stereomicroscope, and then morphometric identification was carried out using a compound microscope (Olympus CX33) at 400x magnification.

Egg Mass Collection

The inoculum was prepared from previously collected root and soil samples. About 1 kg of tomato roots was cut into 1–2 cm pieces and placed in a test tube containing 2% NaOCl solution [17]. The test tube was shaken for 5 minutes to extract *Meloidogyne* sp. eggs. The extracted eggs were filtered using a 500-mesh sieve and rinsed thoroughly with distilled water to remove any remaining NaOCl. The collected eggs were transferred to a petri dish containing 10 mL of sterile distilled water and incubated in the dark. After 7 days of incubation, the hatched J2 stage *Meloidogyne* sp. was used for further experiments

Purification of Trichoderma sp.

Trichoderma isolates were obtained from the collection of the Plant Health Laboratory, Universitas Pembangunan Nasional Veteran Jawa Timur. The *Trichoderma* isolate used was recorded under the name *Trichoderma* sp. strain K9302. *Trichoderma* sp. was purified by taking *Trichoderma* isolates with a diameter of 10 mm and then growing them on PDA media.

Preparation Biocide of *S. narbonensis* strain PSM242 and *Trichoderma* sp. Combine in Enrich Mediums

The preparation of the biocide involved culturing 10^7 CFU/ml *S. narbonensis* strain PSM242 and *Trichoderma* sp. isolates in 100 mL of Potato Glucose Extract (EKG) medium. The resulting suspensions of *S. narbonensis* strain PSM242 and *Trichoderma* sp. were utilized in the subsequent stage. One milliliter of each suspension was added to 99 mL of EKG medium [18] enriched with additional nutrients. The nutrients used for enrichment were glucose nitrate and coconut water, each at a concentration of 10 ppm.

S. narbonensis strain PSM242 and *Trichoderma* sp. against *Meloidogyne* sp. In Vitro

150 J2 *Meloidogyne* sp. in 4 mL of suspension was put into a 5 cm diameter petri dish. Each suspension of *S. narbonensis* strain PSM242 and *Trich-*

oderma sp. as much as 1 mL was poured into the petri dish according to the treatment. Furthermore, the treated nematodes were incubated for 24 hours at a temperature of 28°C in a dark room. After 24 hours of incubation, the nematode suspension was dripped with 100 L of 1 N NaOH to activate the inactivated nematodes [19; 20]. Active nematodes showed a wavy phenotype, while dead nematodes showed an upright and stiff body position after 5 minutes of administration of 1 N NaOH.

S. narbonensis strain PSM242 and *Trichoderma* sp. against *Meloidogyne* sp. in Land Experiment

The parasitization ability test of the combination of *S. narbonensis* strain PSM242 and *Trichoderma* sp. on several types of production media was conducted at the Plant and Land Health Laboratory, Faculty of Agriculture, Department of Agrotechnology, East Java Veteran National Development University. Cherry tomato seeds were sown in soil and compost media with a ratio of 1:2 using a pot tray for 21-28 days. After that, the plants were transferred into 35 cm x 35 cm polybags containing soil and compost. 3 days after planting (DAP), inoculate $\pm$ 1000 nematode eggs on the roots of cherry tomato plants (Wulandari et al., 2019). Then inoculate *S. narbonensis* strain PSM242 and *Trichoderma* sp. according to the predetermined treatment combination as much as 20 ml. Observations were made in the generative phase to see the inhibition of root nodule formation on the roots, the population level of root nodule nematodes on the roots, and agronomic aspects including root length, root weight, and fruit weight. Nematode population analysis was carried out by extraction-isolation of *Meloidogyne* sp. juvenile phase 2 (J2) using a modified Baerman technique [16].

Data analysis

The experiment used a split-plot design in a factorial randomized block design (RBD), with the first factor being the concentration of *S. narbonensis* strain PSM242 and *Trichoderma* sp. The second factor is the type of culture medium. Data analysis using (ANOVA), and further tested using the DMRT test with 95% confidence level.

Results and discussion

Isolation and identification of Streptomyces strain

Two isolates of *Streptomyces* were obtained from soil samples. Microscopic and macroscopic identification was carried out on the isolates that had been obtained. The morphological characteristics of *Streptomyces* isolates are presented macroscopically in Table 1 and microscopically in Figure 1.

Table 1.

Morphological characteristics of *Streptomyces* isolates

Bacterial strain	Colony morphology					Gram
	Shape	Color	Surface	Edge	Width (mm)	
PSM241	Filamentous	Yellowish	Pulvinate	Filiform	1-2	+
PSM242	Filamentous	White	Convex	Filiform	1-2	+

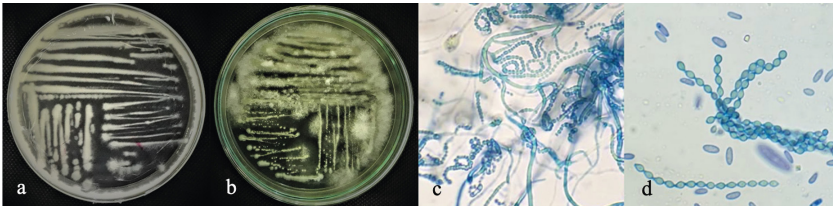


Fig. 1. Morphology characteristic a) macroscopic PSM241; b) macroscopic PSM242; c) hyphae structure of Streptomyces strain; d) spores of Streptomyces strain

The two isolates were identified molecularly based on the 16S rRNA gene. Analysis of the 16S rRNA gene showed that strain PSM241 had 96% similarity to *Streptomyces narbonensis* strain NBRC 12801, and strain PSM242 had 100% similarity to *Streptomyces narbonensis* strain NRRL B-1680 (Table 2, Figure 2). Strain PSM242 was selected and used for biocide formulation.

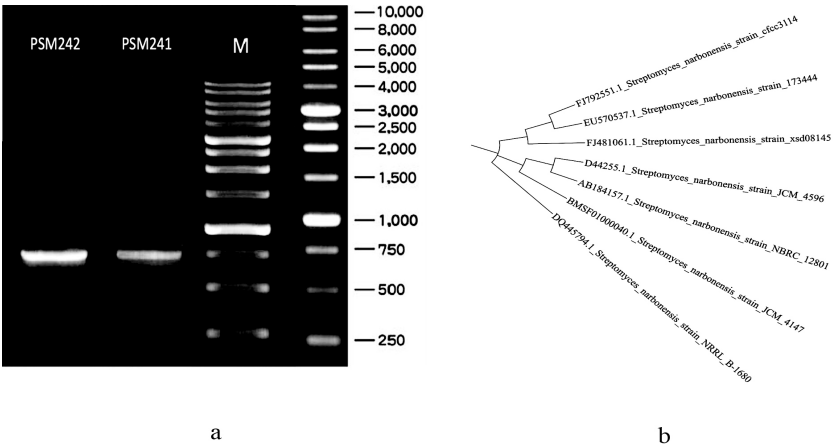


Fig. 2. (a) Visualization of agarose gel electrophoresis amplified the 16S rRNA gene of PSM241 and PSM242 strain (DNA Ladder 10.000 bp), (b) circular phylogenetic tree with Neighbor-Joining Method and Jones-Taylor-Thornton (JTT) substitution model

Table 2.

Identification of *Streptomyces* isolates

Bacterial strain	Homology (%)	Species database on GenBank	Accession no.
PSM241	96	<i>Streptomyces narbonensis</i> strain NBRC 12801	AB184157.1
PSM242	100	<i>Streptomyces narbonensis</i> strain NRRL B-1680	DQ445794.1

Extraction and identification of root-knot nematodes

Morphological observations showed that the nematode eggs were round and elongated. The nematode larvae were elongated and slender with a slender tail. There was a straight stylet and a clear basal knob, the adult male nematode was elongated with a short tail. In contrast, the female nematode had a rounded body shape like a pear, with a short neck and no tail. Adult male nematodes are elongated and longer than females with variations in the length of up to 2 mm, moving slowly, tails are short and rounded, and heads are not notched. The female nematode has a pear-like shape, does not have a tail, and a short neck, more than 0.5 mm long and 0.3 - 0.4 mm wide. In terms of size, female nematodes have a larger diameter than male nematodes (Figure 3). Based on these morphological characteristics, it can be identified that the root knot nematodes found are *Meloidogyne* sp.



Fig. 3. *Meloidogyne* morphological identification a) egg; b) juvenile; c) basal knob of male nematode; d) male infective larval; e) female infective larval

In Vitro Experiment *S. narbonensis* strain PSM242 and *Trichoderma* sp. against *Meloidogyne* sp.

S. narbonensis strain PSM242 and *Trichoderma* sp. were grown in EKG media enriched with glucose nitrate and coconut water. Mortality of adult *Meloidogyne* sp. samples was observed in this in vitro test. The results showed that *S. narbonensis* strain PSM242 and *Trichoderma* sp. grown in enriching EKG have nematicidal ability and successfully kill root-knot nematodes *Meloidogyne* sp. Mortality of *Meloidogyne* sp. in the in vitro test is presented in Figure 4.

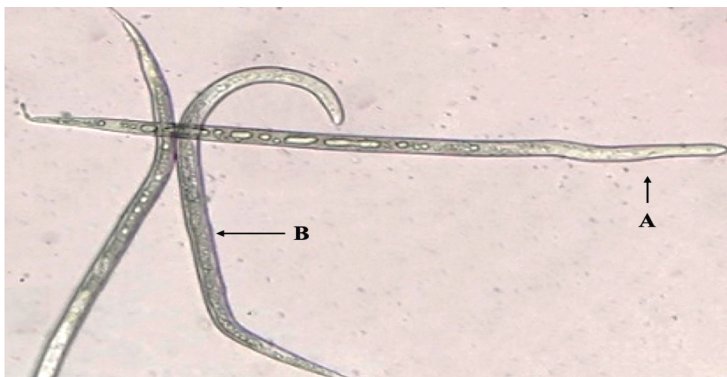


Fig. 4. Microscopic appearance of a) dead body, and b) living body of *Meloidogyne* sp.

Effect of S. narbonensis strain PSM242 and *Trichoderma* sp. against *Meloidogyne* sp.

Quantitatively, gall diameter, gall weight, and population of *Meloidogyne* sp. aged juvenile 2 were calculated to observe the effect of *S. narbonensis* strain PSM242 and *Trichoderma* sp. treatment with a mixture of EKG medium enrichment. The results of these measurements are presented in Table 3.

Table 3.

Effect of *S. narbonensis* strain PSM242 and *Trichoderma* sp. on gall diameters, gall weight, and population of juvenil 2 *Meloidogyne* sp.

Treatments	Gall Diameter (mm)	Gall Weight (g)	J2 Population Average
Control (-) without treatments	9.33 f	74.50 d	247.02 h
Control (+) <i>S. narbonensis</i> + <i>Trichoderma</i> sp.	6.00 d	18.00 b	80.30 de
<i>Trichoderma</i> sp.+ EKG	7.33 e	27.50 c	142.54 g
<i>Trichoderma</i> sp. + EKG + GN	7.00 de	25.80 c	123.97 fg
<i>Trichoderma</i> sp. + EKG + CW	6.67 de	20.03 bc	93.17 e
<i>S. narbonensis</i> + EKG	7.33 e	27.60 c	139.81 g
<i>S. narbonensis</i> + EKG + GN	7.00 de	25.00 c	114.53 f
<i>S. narbonensis</i> + EKG + CW	6.33 d	17.33 b	85.83 de
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG	4.00 b	10.03 ab	21.05 b
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG + GN	4.00 b	9.37 ab	10.86 a
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG + CW	1.33 a	4.30 a	6.10 a
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG	5.67 cd	17.17 b	75.80 d

<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG + GN	5.33 c	15.83 b	51.95 c
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG + CW	5.33 c	14.77 b	30.23 bc

Note: The numbers in the column followed by the same letter indicate that there is no significant difference in the DMRT test at the 95% confidence level.



Fig. 5. Growth of infected cherry tomato roots a) *S. narbonensis* + *Trichoderma* sp. 2:2 + EKG + CW; b) *S. narbonensis* + *Trichoderma* sp. 2:2 + EKG + GN; c) *S. narbonensis* + *Trichoderma* sp. 2:2 + EKG; d) *S. narbonensis* + *Trichoderma* sp. 1:3 + EKG + CW; e) *S. narbonensis* + EKG + CW; f) Control (+) *S. narbonensis* + *Trichoderma* sp.; g) *Trichoderma* sp. + EKG + CW; h) Control (-) without treatments

Table 3 shows the lowest gall diameter, gall weight, and J2 population of *Meloidogyne* sp. were in the *S. narbonensis* + *Trichoderma* sp. 1:3 + EKG + CW treatment, namely with a gall diameter of 1.33 mm, a gall weight of 4.30 g, and an average J2 population of 6.10 root-knot nematodes. The highest results were in the negative control treatment or without any treatment, with a gall diameter of 9.33 mm, a gall weight of 74.50 g, and an average J2 population of 247.02. In general, Table 3 provides information that providing additional nutrients to the EKG medium reduces gall diameter, gall weight, and the average J2 population. When compared to the *S. narbonensis* + *Trichoderma* sp. 1:3 and *S. narbonensis* + *Trichoderma* sp. 2:2 treatments, the lowest values of J2 population, gall weight,

and diameter were possessed by the addition of nutrients to the EKG medium. Meanwhile, when compared between multi-antagonistic *S. narbonensis* + *Trichoderma* sp. with the treatment of *S. narbonensis* or *Trichoderma* sp. individually, it shows that the lowest suppression is possessed by the combined treatment of *S. narbonensis* + *Trichoderma* sp. The observation of the form of gall infection *Meloidogyne* sp. on the roots of cherry tomato plants is presented in Figure 5.

Effect of S. narbonensis strain PSM242 and Trichoderma sp. on Cherry Tomato Plant

The effect of *S. narbonensis* strain PSM242 and *Trichoderma* sp. on the growth of cherry tomato plants was also observed. Data on root length and root weight are presented in Table 4 and data on average fruit weight are presented in Table 5.

Table 4.

Effect of *S. narbonensis* strain PSM242 and *Trichoderma* sp. in enriching media on plant root growth

Treatment	Root Length (cm)	Root Weight (g)
Control (-) without treatments	6.60 a	15.44 a
Control (+) <i>S. narbonensis</i> + <i>Trichoderma</i> sp.	24.27 b	44.13 b
<i>Trichoderma</i> sp.+ EKG	18.27 ab	23.34 a
<i>Trichoderma</i> sp. + EKG + GN	22.60 ab	40.65 b
<i>Trichoderma</i> sp. + EKG + CW	22.80 ab	43.35 b
<i>S. narbonensis</i> + EKG	22.37 ab	23.34 a
<i>S. narbonensis</i> + EKG + GN	20.70 ab	42.88 b
<i>S. narbonensis</i> + EKG + CW	24.27 b	49.93 b
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG	32.69 bc	77.82 c
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG + GN	37.60 bc	117.91 d
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG + CW	42.57 c	165.37 e
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG	23.13 ab	54.52 bc
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG + GN	26.30 bc	54.69 bc
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG + CW	29.30 bc	69.89 c

Note: The numbers in the column followed by the same letter indicate that there is no significant difference in the DMRT test at the 95% confidence level.

Table 4 shows that the longest and heaviest roots were found in the treatment of adding coconut water to the EKG media, namely 42.57 cm and 165.67 g. While the lowest weight and shortest roots were found in the negative control

treatment or without any treatment. When compared with the treatments of *S. narbonensis* + *Trichoderma* sp. 1:3 and *S. narbonensis* + *Trichoderma* sp. 2:2, the highest root length and weight values were possessed by the addition of nutrients to the EKG media. Meanwhile, when comparing the multi antagonist *S. narbonensis* + *Trichoderma* sp. with the treatment of *S. narbonensis* or *Trichoderma* sp. individually, it shows that the highest root growth was possessed by the combined treatment of *S. narbonensis* + *Trichoderma* sp.

Table 5.

Effect of *S. narbonensis* strain PSM242 and *Trichoderma* sp. in enriching media on cherry tomato fruit weight

Treatments	Fruit Weight (g/plant)
Control (-) without treatments	32.86 a
Control (+) <i>S. narbonensis</i> + <i>Trichoderma</i> sp.	127.00 b
<i>Trichoderma</i> sp.+ EKG	116,77 b
<i>Trichoderma</i> sp. + EKG + GN	132,61 b
<i>Trichoderma</i> sp. + EKG + CW	136,00 b
<i>S. narbonensis</i> + EKG	122.33 b
<i>S. narbonensis</i> + EKG + GN	127.80 b
<i>S. narbonensis</i> + EKG + CW	135.30 b
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG	235.76 c
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG + GN	239.52 c
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 2:2 + EKG + CW	241.36 c
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG	212.64 c
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG + GN	220.47 c
<i>S. narbonensis</i> + <i>Trichoderma</i> sp. 1:3 + EKG + CW	225.75 c

Note: The numbers in the column followed by the same letter indicate that there is no significant difference in the DMRT test at the 95% confidence level.

Table 5 shows that the highest fruit weight was in the treatment of *S. narbonensis* + *Trichoderma* sp. 2:2 + EKG + CW with a value of 241.36 g per plant. The second and third highest fruit weights were in the treatment of *S. narbonensis* + *Trichoderma* sp. 2:2 + EKG + GN with a value of 239.52 g, and *S. narbonensis* + *Trichoderma* sp. 2:2 + EKG with a value of 235.76 g. While the lowest weight was in the negative control treatment or without treatment with a value of 32.86 g.

Discussion

Root-knot nematodes cause significant damage to plants, including stunted growth, nutrient deficiencies, leaf browning, premature wilting, root rot, re-

duced photosynthetic pigment levels, and poor fruit quality, ultimately leading to production losses and shortened shelf life [21]. Numerous studies have focused on the biological control of plant-parasitic nematodes, particularly *Meloidogyne* sp. [21; 22; 23].

This study highlights the potential of combining *S. narbonensis* strain PSM242 and *Trichoderma* sp. with enriching EKG culture media as a biological agent to suppress *Meloidogyne* sp. reproduction and inhibit gall formation on the roots of cherry tomato plants. Both *S. narbonensis* strain PSM242 and *Trichoderma* sp. significantly reduced the gall index and the population of J2 *Meloidogyne* sp. in infected plant tissues. Research [24; 25; 26] demonstrated that *T. harzianum* effectively controls *Meloidogyne javanica* by inhibiting reproduction, suppressing gall formation, and enhancing host plant growth. Additionally, the *Streptomyces* group has been shown to delay the nematode's life cycle in root tissues, thereby reducing population levels [7; 26; 27]. Report [7; 28] shows that the combination of *Streptomyces* sp. significantly decreased root nodule formation and nematode population density in tomato seedling roots. Nematicidal actinomycete [29; 30] reduced *Meloidogyne incognita*-induced root nodule disease by 71.93% in chili plants, while also improving plant growth by enhancing shoot and root length, weight, and fruit yield.

Tomato plant roots treated with biological agents exhibited greater weight than untreated roots (Table 4). Colonization by a mixture of *T. harzianum* and *G. virens* nitrates was particularly noticeable at the root base [8]. *Streptomyces* species promote plant growth through the production of indole-3-acetic acid (IAA), which stimulates root growth and the production of siderophores [31; 32]. IAA, a phytohormone produced by nematicidal microorganisms, enhances lateral and adventitious root growth, increasing mineral and nutrient uptake [33; 34]. Additionally, *Streptomyces* sp. improve nutrient availability by producing siderophores and solubilizing phosphate, converting complex soil nutrients into simpler forms for plant uptake [35].

Several types of nutrients play an important role in regulating the production of essential enzymes such as proteases and chitinases, which are essential for controlling nematodes [36]. Protease enzymes, for example, degrade proteins, the main components of nematodes and their eggs. Studies [36; 37] have shown that microbes capable of producing proteases are more effective in controlling *Meloidogyne* sp. compared to those without this ability. Regulation of protease production in microbes depends on the availability of phosphorus, which acts as an effector, and amino acids, which function as inhibitors when protease levels are excessive [38]. Pure coconut water con-

tains phosphorus and amino acids, making it a potential source of nutrients to increase protease activity [39; 40].

Similarly, chitinase enzymes are essential in controlling nematodes, because the body wall and eggs of nematodes contain chitin [41; 42; 43]. The combined activity of proteases and chitinases can degrade the structural integrity of nematodes, leading to their death [36; 44]. The composition of the media significantly affects the effectiveness of *S. narbonensis* strain PSM242 and *Trichoderma* sp. as biological agents. The use of EKG media with additional nutrients in the form of glucose nitrate and coconut water has been shown to support the metabolic processes of *S. narbonensis* strain PSM242 and *Trichoderma* sp. according to the literature study.

The results of the observation showed that the combination of *S. narbonensis* strain PSM242 and *Trichoderma* sp. with the appropriate culture media effectively inhibited nematode attacks and increased the growth of plants attacked by root-knot nematodes (Table 3). This treatment significantly increased plant production. Studies have reported that tomato seedling roots treated with *T. harzianum* and *Streptomyces* sp. developed root branches within 24 hours after inoculation [8; 45]. In addition, the administration of *Trichoderma* sp. and *Streptomyces* sp. increased cherry tomato production compared to untreated plants. This increase was due to the production of the root growth hormone IAA by *Trichoderma* sp. and *Streptomyces* sp., which promoted root development and fruit yield [46; 47; 48]. Bioagents such as *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Trichoderma* are effective against root-knot nematodes, increasing healthy tomato fruit yield by 19%–66% [49; 50; 51].

Conclusion

The study successfully isolated *Streptomyces narbonensis* strain PSM242 and *Trichoderma* sp. from soil and demonstrated their effectiveness as biocontrol agents against root-knot nematodes (*Meloidogyne* sp.). In vitro experiments indicated significant nematicidal activity, with the combination treatment resulting in the lowest gall diameter (1.33 mm), gall weight (4.30 g), and juvenile nematode populations (6.10). Additionally, treated cherry tomato plants exhibited enhanced growth, with the longest roots measuring 42.57 cm and the highest fruit weight reaching 165.37 g. These findings support the potential of using microbial antagonists for sustainable agricultural pest management.

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