

Article

Integrated Control of Tomato Damping-Off Using *Stenotrophomonas rhizophila* and CuO Nanoparticles

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Abstract

Damping-off caused by *Fusarium* spp. limits tomato seedling establishment, while chemical control is constrained by resistance development and environmental risks. As a result, biological alternatives and nanomaterials have attracted increasing interest. This study aimed to quantify the in vitro inhibition and in vivo control of *Fusarium* spp. associated with tomato damping-off using the marine strains KN1 and KN2 of *Stenotrophomonas rhizophila* and copper oxide nanoparticles (CuO-NPs). Nine fungal isolates were recovered from symptomatic plants; the most virulent isolate (3DR23HA) caused 60% disease incidence and was identified as *Fusarium oxysporum*. In dual-culture assays, both bacterial strains inhibited mycelial growth, with percentage inhibition of radial growth (PIRG) values exceeding 65% in several isolates, whereas KN1 showed greater inhibition of conidial germination. CuO-NPs exhibited a concentration-dependent response, reaching near-complete suppression at 0.75–1.0 mg mL^{−1}. In seedlings, the inoculated control showed 100% incidence and a disease severity index (DSI) of 85%, whereas KN1, KN1 + CuO-NPs at 0.75 mg mL^{−1}, and KN2 + CuO-NPs at 0.75 mg mL^{−1} achieved a DSI of 0 and 100% control efficiency, while also improving growth and biomass. Overall, *S. rhizophila*, particularly strain KN1, and CuO-NPs at 0.75 mg mL^{−1} represent a promising strategy for the integrated management of tomato damping-off in nurseries.

Keywords: *Fusarium oxysporum*; marine bacteria; saladette tomato; biocontrol



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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide. Recent FAO statistics indicate that, in 2024, global production reached 1.2 billion tonnes of vegetables and 954 million tonnes of fruits, with tomato accounting for 188 million tonnes [1]. Given the global importance of tomato cultivation, constraints affecting seedling establishment can compromise crop uniformity, yield, and economic returns. In this context, damping-off, commonly associated with soil-borne

phytopathogenic fungi such as *Fusarium* spp., is one of the most important diseases affecting tomato because it can severely reduce seedling emergence and stand establishment, with consequent effects on crop uniformity and productivity [2,3]. Because the disease occurs during germination and early seedling development, it increases the need for re-sowing or re-transplanting and further aggravates production losses [4]. In commercial nurseries, high humidity, dense seedling trays, and continuous production cycles create favorable microclimatic conditions for infection, thereby increasing the economic impact of the disease [5,6]. Management of damping-off still relies largely on synthetic fungicides; however, their repeated use can favor the selection of resistant pathogen populations, contribute to environmental contamination, and pose risks to human and animal health [7,8]. These limitations support the evaluation of alternative strategies that are effective against soil-borne phytopathogens and compatible with sustainable nursery production systems.

Biological control has emerged as a relevant alternative for suppressing phytopathogens through the use of beneficial microorganisms, mainly bacteria and fungi [9,10]. In this context, marine bacteria represent an important source of antimicrobial compounds, as adaptation to saline and fluctuating environments may confer distinctive physiological and metabolic traits that favor the production of bioactive metabolites with potential for plant disease suppression [11,12]. The use of marine bacteria as biocontrol agents has reduced several plant diseases in crops such as mango [13], melon [14], strawberry [15], and chickpea [16], highlighting genera such as *Bacillus* [17], *Stenotrophomonas* [18], and *Micrococcus* [19]. Their antagonistic activity against phytopathogens has been demonstrated both in vitro and in vivo through multiple mechanisms, including competition for space and nutrients, siderophore production, biofilm formation, secretion of hydrolytic enzymes, and induction of host resistance [20–22]. Collectively, this evidence provides a scientific basis for exploring marine bacterial strains as biological agents against damping-off-associated phytopathogens in tomato.

Among these bacterial taxa, *Stenotrophomonas rhizophila*, a plant-associated species within the genus *Stenotrophomonas* of the phylum *Pseudomonadota*, has emerged as a promising bacterium for damping-off management because of its ability to colonize the rhizosphere and persist under competitive soil conditions [23–25]. This ecological competence is particularly relevant in damping-off pathosystems, in which disease establishment is largely determined during the earliest stages of seed and root colonization; infection of the seed or emerging radicle may result in pre-emergence damping-off, whereas colonization of the hypocotyl or root-collar region after emergence may cause water-soaked lesions, tissue constriction, and seedling collapse [2,5,26]. Therefore, evaluating a marine strain of *S. rhizophila* is biologically justified, as its rhizosphere competence and multiple antagonistic mechanisms may restrict phytopathogen establishment during the most susceptible phase of seedling development, both in vitro and under nursery conditions.

In parallel, nanomaterials have gained increasing attention as tools for plant disease management because of their distinctive physicochemical and biological properties [27,28]. Among metallic nanoparticles with antimicrobial activity, copper-based nanoparticles are particularly relevant because of their relatively low cost, high surface reactivity, and reported efficacy against a broad spectrum of phytopathogen [29–32]. In addition to their antimicrobial potential, copper is an essential micronutrient involved in plant growth and in the function of proteins associated with defense responses, such as plastocyanin, peroxidases, and oxidases [33,34]. Although the precise antimicrobial mechanism of copper oxide nanoparticles (CuO-NPs) remains under investigation, available evidence suggests that their activity may involve reactive oxygen species

(ROS) generation and damage to essential cellular targets, including deoxyribonucleic acid (DNA) [35,36]. However, their efficacy is strongly influenced by dose, formulation, and exposure route [37,38]. For example, in tomato plants challenged with *Fusarium oxysporum* f. sp. *lycopersici*, chitosan-loaded CuO nanoparticles applied at 1 mg L⁻¹ reduced *Fusarium* wilt severity by 91.5% after 75 days, whereas applications at 25 and 50 mg L⁻¹ reduced disease severity by 96.0% and 98.0%, respectively, outperforming the conventional fungicide Kocide 2000, which achieved about 90% disease reduction at 2.5 g L⁻¹ [39]. This aspect is particularly relevant in nursery systems, where CuO-NPs may be applied as foliar sprays [40,41]. When applied as foliar sprays, copper-based nanoparticles may also act as elicitors of plant defense responses. These effects have been associated with enhanced antioxidant capacity, including the upregulation or activation of superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione peroxidase (GPX); stimulation of the phenylpropanoid pathway, including increased phenylalanine ammonia-lyase (PAL) and, in some cases, polyphenol oxidase (PPO) activity; and induction of pathogenesis-related (PR) genes [42–45]. Therefore, defining effective foliar CuO-NP application strategies under nursery conditions is relevant for their practical use against damping-off-associated phytopathogens.

From a phytopathological perspective, integrating microbial antagonists with nanomaterials represents a rational strategy because both components may operate through complementary mechanisms. Beneficial bacteria can reduce phytopathogen establishment at the root-substrate interface through ecological competition and metabolite- or enzyme-mediated inhibition [20,21], whereas foliar-applied CuO-NPs may contribute through direct antimicrobial activity and by enhancing host defense responses [38,42,43]. Thus, combining a rhizosphere-competent antagonistic bacterium with a nanoparticle-based treatment may provide broader protection than either component applied individually. Despite this conceptual and practical relevance, evidence on the combined use of *Stenotrophomonas rhizophila* and CuO-NPs against *Fusarium*-associated damping-off in tomato seedlings remains limited. Therefore, the objective of this work was to evaluate the *in vitro* and *in vivo* effects of the marine bacterium *Stenotrophomonas rhizophila* and foliar-applied copper oxide nanoparticles (CuO-NPs) on the suppression of *Fusarium* spp. associated with damping-off in tomato seedlings, and to determine whether the integration of both components could improve disease suppression compared with their individual application.

2. Materials and Methods

2.1. Plant Material and Isolation of Phytopathogens

Symptomatic saladette tomato plants (cv. Río Grande) showing stem base rot and/or root rot were collected on 7 March 2023 from a commercial field in Baja California Sur, Mexico (23°47'00" N, 110°17'40" W) (Figure 1). Samples were individually labeled, placed in polyethylene bags, transported in a cooler, and processed upon arrival at the Microbial Biotechnology Laboratory (CIBNOR). Diseased tissues were rinsed under running water, and small fragments were excised from the lesion margins, including the transition zone between symptomatic and healthy tissue. The fragments were surface-disinfected with 3% sodium hypochlorite for 30 s, rinsed three times with sterile distilled water, blotted dry on sterile paper, and plated onto potato dextrose agar (PDA) amended with Bactrol® (BASF Mexicana, Ciudad de México, Mexico) at 0.5 g L⁻¹ to suppress bacterial growth. Plates were incubated at 28 °C and monitored until fungal growth emerged. Hyphal tips from representative colonies were transferred to fresh PDA plates to obtain pure cultures and incubated at 28 °C for 7 days [21]. Each recovered

fungus isolate was then assigned an internal alphanumeric code (e.g., 3DR23HA, 3DR23, 12DR23, 11R23, and 05DR23) for laboratory tracking and subsequent analyses.

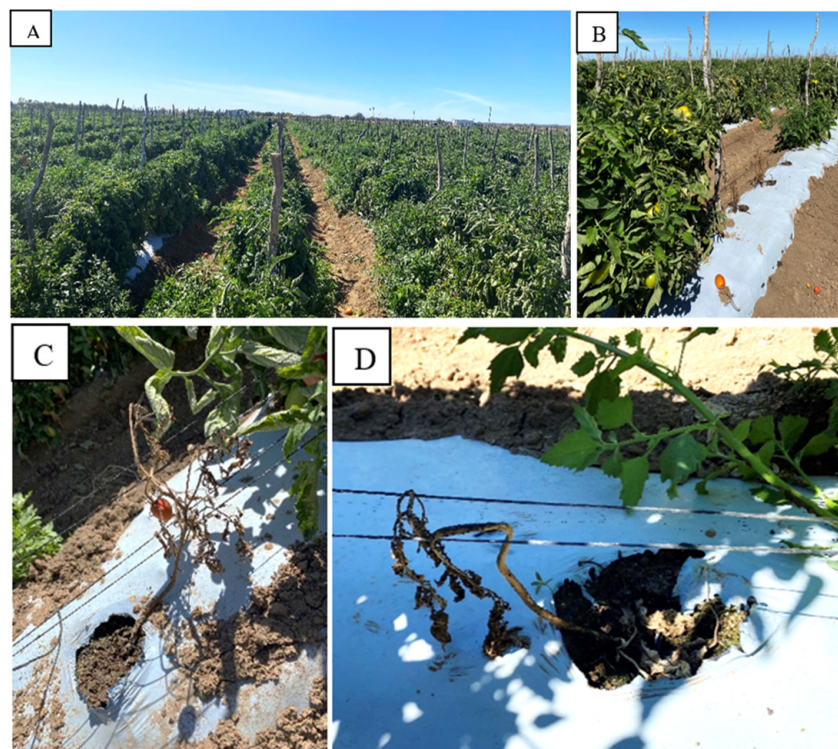


Figure 1. Symptomatic saladette tomato plants (cv. Río Grande) collected from a commercial field in Baja California Sur, Mexico. (A) View of “El Chilerito” Ranch. (B–D) Plants showing stem base and root rot symptoms.

2.2. Morphological Identification of *Fusarium* spp. Isolates

Morphological identification of *Fusarium* spp. isolates was performed based on the taxonomic keys and diagnostic criteria described by Summerell et al. [46]. Pure cultures were grown on PDA at 28 °C for 7 days under a 12 h light/12 h dark photoperiod to promote sporulation. Macroscopic traits were recorded, including colony pigmentation (surface and reverse), texture, and margin characteristics. For microscopic examination, sporulating structures were mounted in lactophenol cotton blue and observed under a light microscope at 400×–1000× magnification. The diagnostic characters evaluated included the morphology of macroconidia and microconidia, as well as the presence and distribution of chlamydospores [46].

2.3. Pathogenicity Test

Each fungal isolate was cultured on PDA at 28 °C for 7 days. Conidia were harvested by flooding the plate surface with sterile distilled water and gently scraping the colony to dislodge them. The resulting suspension was filtered through sterile gauze to remove mycelial fragments, and the conidial concentration was adjusted to 1×10^4 conidia mL^{−1} using a hemocytometer. Tomato seedlings (cv. Río Grande) at the 3–4 true-leaf stage, with no visible symptoms of damping-off or root rot at the time of inoculation, were inoculated by applying 5 mL of each conidial suspension as a soil drench around the root zone. Control plants received 5 mL of sterile distilled water following the same procedure. Plants were arranged in a completely randomized design with fifteen seedlings per isolate ($n = 15$) and maintained in an environmental chamber for 20 days at 28 ± 2 °C and 70% relative humidity under a 12 h light/12 h dark photoperiod.

Disease severity was assessed using a modified ordinal scale based on Boehm et al. [47], adapted for post-emergence damping-off in transplanted tomato seedlings. Because the assay was conducted with seedlings at the 3–4 true-leaf stage, pre-emergence damping-off was not applicable under these conditions. Disease severity was scored as follows: 0 = symptomless seedlings; 1 = mild symptoms (wilting, chlorosis, and/or small lesions at the hypocotyl/root-collar region, without collapse); 2 = moderate symptoms (evident wilting, chlorosis, and/or lesions accompanied by noticeable growth reduction, with seedlings still remaining upright); 3 = severe symptoms (pronounced wilting and/or extensive lesions associated with marked growth reduction, with seedlings still remaining upright); and 4 = post-emergence damping-off (seedling collapse or death after emergence).

Disease incidence (DI, %) was calculated as the proportion of plants showing visible symptoms consistent with post-emergence damping-off (e.g., chlorosis, wilting, lesions in the hypocotyl/root-collar region, or stunting) relative to the total number of evaluated plants, using the following expression:

$$DI (\%) = (FI/TF) \times 100$$

where *FI* is the number of infected plants and *TF* is the total number of plants [48].

At the end of the experiment, roots were gently washed to remove the substrate, and root fresh weight (g) was recorded using a precision balance (OHAUS Adventurer® AX423N, OHAUS Corp., Parsippany, NJ, USA; ± 0.002 g). Root dry weight (g) was determined after oven-drying at 60 °C to constant mass in a benchtop drying oven (Model 9025H). To fulfill Koch's postulates, the phytopathogen was re-isolated from symptomatic collar and root tissues onto PDA, purified, and identified by comparing its colony and microscopic morphology with those of the original isolate [49].

2.4. Molecular Identification and Phylogenetic Analysis

All nine isolates were initially characterized morphologically and evaluated in the pathogenicity assay. Because isolate 3DR23HA showed the highest disease incidence and was considered the most virulent isolate, it was selected for molecular identification. Genomic DNA was extracted following the method of Raeder and Broda [50]. The ITS1–5.8S–ITS2 rDNA region was amplified using primers ITS1 (5'-TCCGTAGGTGAACCCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR amplification was performed in a thermal cycler (GeneAmp® PCR System 9700, Applied Biosystems, Foster City, CA, USA) with an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were sequenced by MACROGEN (Seoul, Republic of Korea), and the resulting ITS sequence of isolate 3DR23HA was deposited in the NCBI GenBank database under accession number PZ256382.

Reference ITS sequences were retrieved from the NCBI GenBank database and aligned with the obtained sequence using MUSCLE v. 3.8.31 [51]. The multiple alignment was curated using the Gblocks program v. 0.91b [52], implemented in the Phylogeny.fr platform [53], to remove highly variable positions and gap-containing regions while retaining conserved blocks. Based on the curated alignment, the best-fit nucleotide substitution model was determined in MEGA v. 12.0 (MEGA Software, Tempe, AZ, USA) [54]. Phylogenetic analysis was then performed in MEGA v. 12.0 using the Maximum Likelihood (ML) method under the Kimura 2-parameter model with gamma-distributed rate variation among sites (K2P + G). Branch support was assessed by bootstrap analysis with 10,000 replicates. Molecular identification was established based on the phylogenetic clustering of isolate 3DR23HA with reference sequences retrieved from the NCBI GenBank database.

2.5. Bacterial Strains

Marine strains KN1 and KN2 of *Stenotrophomonas rhizophila* were provided by the Microbial Biotechnology Laboratory at CIBNOR. Each strain was cultured in tryptic soy broth (TSB) at 28 °C for 24 h with shaking at 200 rpm. Cell density was adjusted spectrophotometrically to an OD₆₆₀ of 1.0, corresponding to approximately 1×10^8 CFU mL⁻¹.

2.6. In Vitro Antifungal Activity of Marine Bacteria

Dual-culture assays were performed on PDA plates. A 5 mm mycelial plug taken from the actively growing margin of each 7-day-old fungal culture was placed at the center of each Petri dish. Bacterial suspensions were spot-inoculated by depositing 5 µL at a distance of 2 cm from the fungal plug. Control plates received only the fungal plug. Ten replicate plates were prepared for each bacterial strain–fungal isolate combination. Plates were incubated at 28 °C for 7 days. Fungal radial growth (mm) was measured along two perpendicular axes and averaged. The percentage inhibition of radial growth (PIRG) [55] was calculated as follows:

$$\text{PIRG (\%)} = ((R_c - R_t)/R_c) \times 100,$$

where R_c is the mean radial growth of the control colony and R_t is the mean radial growth of the treated colony.

2.7. Spore Germination Inhibition Assay

Spore germination inhibition was evaluated using conidial suspensions of four *Fusarium* spp. isolates (3DR23, 3DR23HA, 4DR23, and 12DR23), each adjusted to 1×10^4 conidia mL⁻¹. Equal volumes of each conidial suspension and each bacterial suspension (1×10^8 cells mL⁻¹) were mixed (1:1, v/v) in sterile microtubes and incubated at 28 °C for 12 h. A control treatment consisted of conidia mixed with sterile distilled water under the same conditions. After incubation, an aliquot of each mixture was placed on a microscope slide, and at least 100 conidia were examined per replicate under a light microscope at 400× magnification. Conidia were considered germinated when the germ tube length was equal to or greater than the conidial length [56]. Spore germination (%) was calculated as follows:

$$\text{Germination (\%)} = (\text{number of germinated conidia} / \text{total conidia counted}) \times 100.$$

Spore germination inhibition (%) was calculated relative to the control as:

$$\text{Inhibition (\%)} = ((G_c - G_t)/G_c) \times 100,$$

where G_c is germination in the control and G_t is germination in the bacterial treatment.

2.8. In Vitro Antifungal Activity of Nanoparticles

Copper oxide nanoparticles (CuO-NPs) were purchased from Sigma-Aldrich/Aldrich (St. Louis, MO, USA; Product No. 544868; CAS No. 1317-38-0). According to the supplier's specifications, the material was provided as a black nanopowder with a nominal particle size of <50 nm. The certificate of analysis for the product lot used in this study reported an average particle size of 40 nm as determined by transmission electron microscopy (TEM), X-ray diffraction consistent with the expected CuO structure, and a copper content of 77.2%. CuO-NPs were evaluated at concentrations of 0.1, 0.25, 0.5, 0.75, and 1.0 mg mL⁻¹. A stock suspension of CuO-NPs was prepared in sterile distilled water and dispersed by sonication in three 15 min cycles (45 min total). The nanoparticle

suspensions were added directly to molten PDA (50–55 °C), thoroughly homogenized, and poured into Petri dishes. Control plates contained PDA without CuO-NPs. A 5 mm mycelial plug taken from the actively growing margin of each fungal isolate grown on PDA for 7 days was placed at the center of each plate. Plates were incubated at 28 °C for 7 days. Ten replicate plates were prepared for each concentration–isolate combination. Radial growth (mm) was measured along two perpendicular axes and averaged. The percentage inhibition of radial growth (PIRG) was calculated using the same equation described in Section 2.6, where R_c corresponds to the mean radial growth of the control colony (PDA without CuO-NPs) and R_t corresponds to the mean radial growth of the treated colony (PDA amended with CuO-NPs) [57].

2.9. In Vivo Experiment

Tomato seedlings at the 3–4 true-leaf stage were transplanted into pots containing 500 g of a sterilized substrate mixture of soil, sand, and vermiculite (2:1:1, $v/v/v$). The substrate was sterilized by autoclaving at 121 °C and 15 psi for 60 min on two consecutive days. Seedlings were inoculated with isolate 3DR23HA (*Fusarium oxysporum*) by applying 5 mL of a conidial suspension (1×10^4 conidia mL^{-1}) as a soil drench around the root zone.

The experiment was conducted under phytopathogen challenge in a completely randomized design with a 3×3 factorial arrangement. The factors were bacterial treatment (none, KN1, and KN2) and CuO-NP concentration (0, 0.50, and 0.75 mg mL^{-1}), including their interaction. For CuO-NP treatments, either alone or in combination with bacteria, suspensions prepared in sterile water were applied as a foliar spray 24 h after phytopathogen inoculation until the foliage of each seedling was uniformly wetted. For bacterial treatments, either alone or in combination with CuO-NPs, 1 mL of each *Stenotrophomonas rhizophila* suspension (1×10^8 cells mL^{-1}) was applied to the stem base (root-collar region) at the soil line. In addition, a synthetic fungicide reference treatment was included, consisting of phytopathogen-inoculated plants treated with carbendazim at 2 mL kg^{-1} of substrate. Each treatment included 15 seedlings ($n = 15$). Pots were maintained in an environmental chamber for 15 days at 28 ± 2 °C and 70% relative humidity under a 12 h light/12 h dark photoperiod.

At 15 days after treatment application, morphological and disease-related variables were recorded for each seedling. Stem length (cm) was measured from the substrate surface to the apical meristem using a graduated ruler. Root length (cm) was measured from the root-collar region to the tip of the main root after gently washing the root system to remove adhering substrate. Stem diameter (mm) was measured at the soil line using a digital caliper. The number of leaves was recorded as the total number of fully expanded true leaves per plant. Fresh biomass (g) was determined as the total fresh weight of each plant immediately after harvest using an analytical balance (OHAUS Adventurer® AX423N; ± 0.002 g). Disease incidence (DI, %) was calculated using the equation described in Section 2.3. Disease severity (DS) was assessed using the ordinal scale described in Section 2.3, adapted from Boehm et al. [47]. Disease severity was expressed as the disease severity index (DSI, %), calculated as:

$$\text{DSI}(\%) = \left[\sum_{i=0}^4 ni(si) / (N \times K) \right] \times 100$$

where ni is the number of seedlings in severity class i , si is the numerical value assigned to class i , N is the total number of assessed seedlings per treatment, and K is the maximum severity score (4).

2.10. Control Efficiency (Abbott, %)

Control efficiency (E, %) was calculated from final disease severity, expressed as the disease severity index (DSI, %), using the Abbott formula [58]. The inoculated control treatment was used solely as the reference for calculating E:

$$E(\%) = ((DSI_{control} - DSI_{treatment}) / DSI_{control}) \times 100$$

where $DSI_{control}$ is the mean DSI (%) of the inoculated control (3DR23HA) and $DSI_{treatment}$ is the mean DSI (%) of each treatment. For statistical comparisons of control efficiency among treatments, the reference control was excluded from the analysis.

2.11. Statistical Analysis

All statistical analyses were performed in R (version 4.4.2). For the in vitro assays, radial growth inhibition (PIRG, %) in the dual-culture test was evaluated using a two-way model with bacterial strain (KN01, KN02), fungal isolate (3DR23, 3DR23HA, 4DR23, and 12DR23), and their interaction as fixed effects; when the interaction was significant, simple-effects pairwise contrasts were conducted and p -values were adjusted using the Holm method ($p \leq 0.05$). For CuO-NP dose–response assays within each *Fusarium* spp. isolate (0.1 – 1 mg mL^{-1}), treatment effects were tested using the Kruskal–Wallis test, followed, when significant, by Dunn’s post hoc test with Holm adjustment ($p \leq 0.05$). For seedling responses, growth variables were analyzed under a CRD with a 3×3 factorial arrangement (bacterial treatment: none, KN1, KN2); CuO-NPs concentration (0 , 0.50 , 0.75 mg mL^{-1}), including the $A \times B$ interaction; because several variables did not meet parametric assumptions, factorial effects were evaluated using the Scheirer–Ray–Hare two-way nonparametric ANOVA on ranks, and significant interactions were decomposed via simple-effects analyses (Kruskal–Wallis) with Dunn–Holm multiple comparisons ($p \leq 0.05$), whereas the synthetic fungicide was treated as a non-factorial reference and compared separately using rank-based pairwise tests with Holm adjustment. Disease incidence (severity > 0) was analyzed as a binary outcome, reported as x/n (%) with 95% Wilson confidence intervals, and factorial effects were assessed using Firth-corrected binomial logistic regression. Disease severity was analyzed using Scheirer–Ray–Hare, with significant interactions followed by simple-effects Dunn–Holm comparisons ($p \leq 0.05$), and severity was additionally summarized as DSI (%). Control efficiency (Abbott, %) was compared using the same factorial framework (Scheirer–Ray–Hare with simple effects and Dunn–Holm, $p \leq 0.05$).

3. Results

3.1. Isolation, Morphological Characterization, and Pathogenicity of Fungal Isolates

Nine fungal isolates were recovered on PDA from tomato plants exhibiting damping-off symptoms. After incubation on PDA at 28°C for 7 days under a 12 h light/12 h dark photoperiod, colonies showed abundant aerial mycelium with a cottony to floccose texture and variable pigmentation among isolates (Figure 2). During early growth, colonies were predominantly white to pale pink; as incubation progressed, some isolates developed more intense pigmentation, ranging from pinkish-violet to brownish, often concentrated in the central zone, whereas others remained cream to whitish with a paler center. Colony margins were generally entire to slightly undulate, and reverse pigmentation ranged from pale yellow to pinkish-violet or brown.

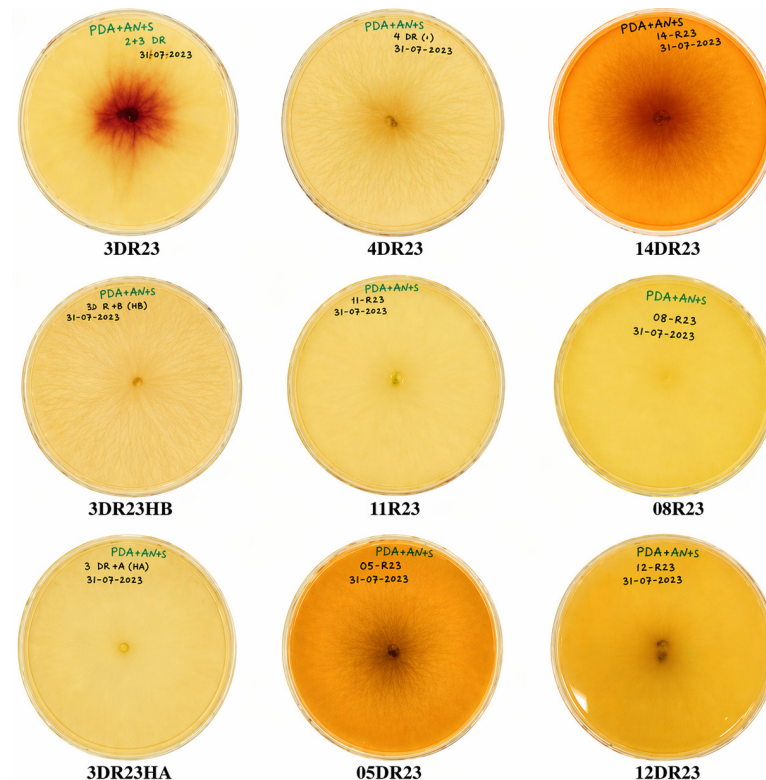


Figure 2. Isolates of *Fusarium* spp. recovered from tomato plants with symptoms of damping-off. Colonies were cultured on PDA and photographed in 90 mm-diameter Petri dishes. Alphanumeric designations correspond to internal isolate codes assigned during fungal isolation and laboratory tracking.

In the microscopic characterization, lactophenol cotton blue-stained preparations revealed sporulating structures consistent with *Fusarium* spp. Hyaline macroconidia were observed, characterized by an elongated, slightly curved shape and evident septation (Figure 3A), together with hyaline microconidia of ovoid to ellipsoidal shape, grouped in masses around hyphae or conidiophores (Figure 3B,C). These macroscopic and microscopic features supported the assignment of the isolates to the genus *Fusarium*.

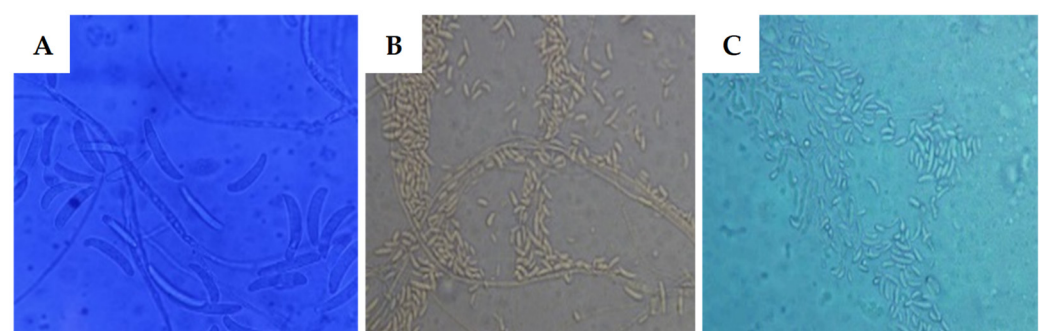


Figure 3. Microscopic structures of *Fusarium* spp. isolates from tomato plants with symptoms of stem softening and/or root rot (damping-off). (A) Macroconidia isolate 11R23. (B,C) Microconidia isolates 12DR23 and 05DR23, respectively. Alphanumeric designations correspond to internal isolate codes assigned during fungal isolation and laboratory tracking. Observations were made at 400× magnification.

The highest observed disease incidence in tomato seedlings was recorded for isolate 3DR23HA (60%), followed by isolates 3DR23 and 12DR23, each with 50% (Table 1). The lowest incidence was observed for isolates 05DR23 and 08R23 (10% each). Symptom

severity was similar among most isolates, which were classified as severity grade 3, whereas isolate 05DR23 produced the lowest severity response (grade 2).

Table 1. Pathogenicity of *Fusarium* spp. isolates and biomass of diseased tomato seedlings with damping off.

Isolate	Disease		Root Biomass	
	Incidence (%)	Severity	Fresh (g)	Dry (g)
3DR23	50	3	3.76 ± 0.51 ^{bc†}	1.81 ± 0.07 ^c
3DR23HA	60	3	3.60 ± 0.69 ^{bc}	1.63 ± 0.12 ^c
3DR23HB	30	3	3.68 ± 0.58 ^{bc}	1.81 ± 0.22 ^c
4DR23	40	3	3.63 ± 0.62 ^{bc}	1.77 ± 0.20 ^c
05DR23	10	2	4.44 ± 0.26 ^{ab}	2.28 ± 0.34 ^b
12DR23	50	3	3.46 ± 0.95 ^c	1.64 ± 0.24 ^c
14DR23	30	3	3.66 ± 0.71 ^{bc}	1.63 ± 0.12 ^c
08R23	10	3	3.82 ± 0.53 ^{bc}	1.74 ± 0.14 ^c
11R23	20	3	3.75 ± 0.38 ^{bc}	1.73 ± 0.39 ^c
Control	-	-	4.79 ± 0.35 ^a	2.90 ± 0.36 ^a

[†] Means with the same letter within each column are statistically equal (Tukey, $p \leq 0.05$), ± Standard deviation.

Regarding root biomass, inoculation reduced root fresh weight relative to the non-inoculated control (4.79 g), with the greatest reduction caused by isolate 12DR23 (3.46 g; 27.8%), followed by isolates 3DR23HA (3.60 g; 24.8%) and 4DR23 (3.63 g; 24.2%). Root dry weight also decreased relative to the control (2.90 g), with the largest reductions recorded for isolates 3DR23HA and 14DR23 (1.63 g; 43.8%) and 12DR23 (1.64 g; 43.4%). The phytopathogen was re-isolated from symptomatic seedlings, thereby supporting Koch's postulates.

3.2. Molecular Identification and Phylogenetic Analysis of Isolate 3DR23HA

The ITS rDNA sequence generated for isolate 3DR23HA was deposited in the NCBI GenBank database under accession number PZ256382. BLASTn (v. 2.17.0+) analysis against the NCBI GenBank database showed that its best match was *Fusarium oxysporum* (accession OR198077.1), with 100% query coverage and 99.02% nucleotide identity. Consistently, in the ITS-based phylogenetic analysis (Figure 4), isolate 3DR23HA (GenBank accession PZ256382; ▲) clustered within the *F. oxysporum* clade together with OR198077.1, with strong bootstrap support (100%), and was clearly separated from clades comprising other *Fusarium* species included in the analysis.

3.3. In Vitro Antifungal Activity of *Stenotrophomonas rhizophila*

Dual-culture assays showed that both marine *Stenotrophomonas rhizophila* strains inhibited the *Fusarium* spp. isolates in vitro, although the magnitude of inhibition depended on the bacterial strain and the fungal isolate (Figures 5 and 6). Regarding mycelial growth, inhibition of radial growth (PIRG, %) exceeded 65% for isolates 3DR23, 3DR23HA, and 4DR23 with both KN1 and KN2, with no significant differences between strains within these isolates (Holm-adjusted $p > 0.05$; Figure 5A). In contrast, for isolate 12DR23, KN1 produced a higher PIRG than KN2 (Holm-adjusted $p \leq 0.05$; Figures 5A and 6).

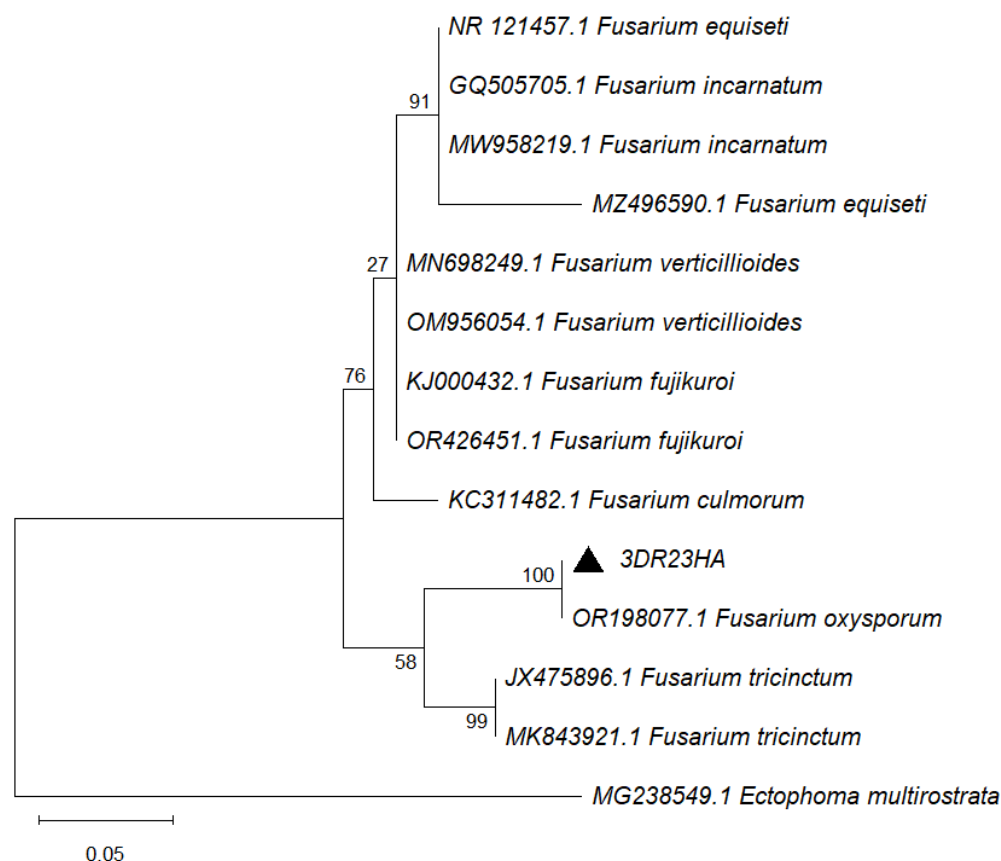


Figure 4. Phylogenetic tree based on ITS rDNA sequences of *Fusarium* spp., showing the placement of isolate 3DR23HA from this study (GenBank accession PZ256382; ▲). Numbers at the nodes indicate bootstrap support values (%) computed from 10,000 replicates. GenBank accession numbers are shown before each species name. *Ectophoma multirostrata* was used as the outgroup.

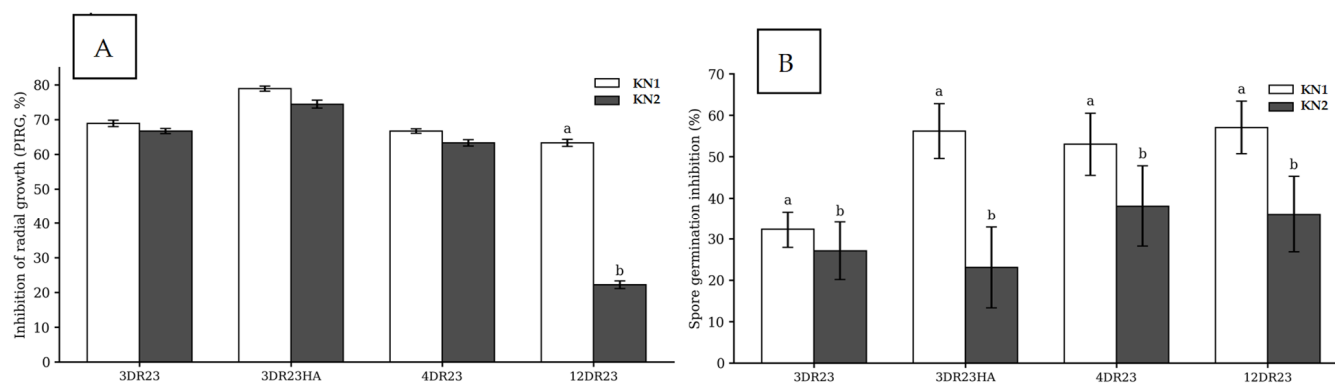


Figure 5. Antagonistic activity of marine *Stenotrophomonas rhizophila* strains (KN1 and KN2) against four *Fusarium* spp. isolates (3DR23, 3DR23HA, 4DR23, and 12DR23). (A) Inhibition of radial growth (PIRG, %). (B) Spore germination inhibition (%). Within each isolate, different lowercase letters indicate significant differences between bacterial strains (KN1 vs. KN2) according to simple-effects pairwise contrasts with Holm-adjusted $p \leq 0.05$. Bars without letters indicate that no significant differences were detected between strains within that isolate. Data are shown as mean $\pm$ standard deviation.

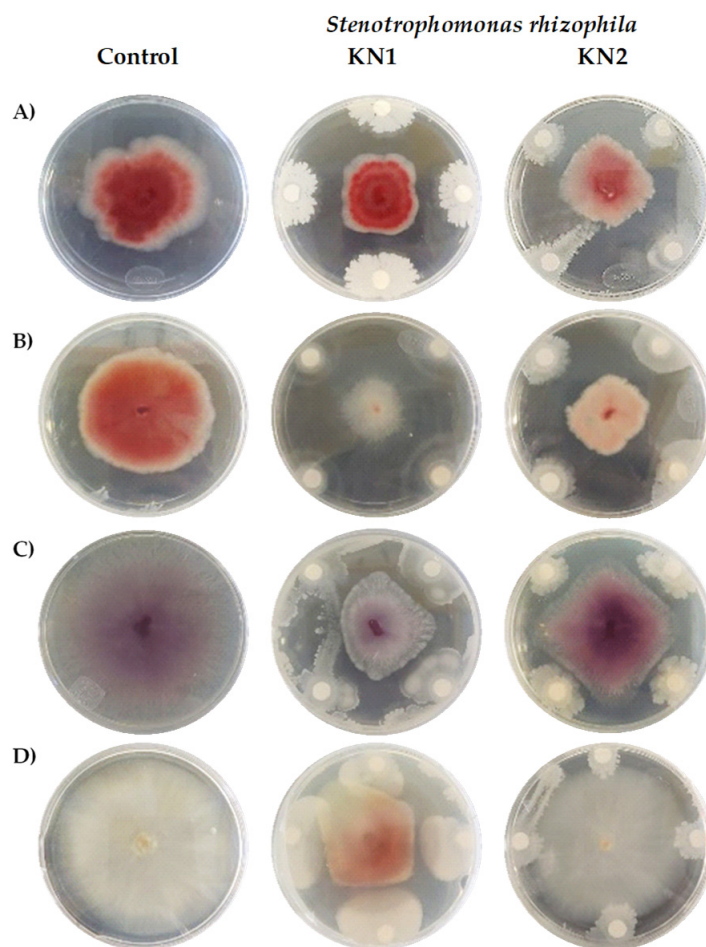


Figure 6. In vitro inhibition of *Fusarium* spp. isolates by two marine strains of *Stenotrophomonas rhizophila* (KN1 and KN2). (A) 3DR23. (B) 3DR23HA. (C) 4DR23. (D) 12DR23.

When spore germination was assessed, KN1 significantly inhibited conidial germination compared with KN2 across all four isolates (3DR23, 3DR23HA, 4DR23, and 12DR23) (Holm-adjusted $p \leq 0.05$; Figure 5B). Overall, these results indicate that KN1 exhibits stronger antagonistic activity than KN2, particularly by suppressing early developmental stages (spore germination) and, in the case of isolate 12DR23, also by restricting mycelial growth.

3.4. In Vitro Antifungal Activity of CuO-NPs

Copper oxide nanoparticles (CuO-NPs) inhibited mycelial growth of all four *Fusarium* spp. isolates in a concentration-dependent manner (Figures 7 and 8). At the lowest concentrations ($0.1\text{--}0.25\text{ mg mL}^{-1}$), inhibition was minimal, whereas increasing the concentration to 0.5 mg mL^{-1} resulted in significantly higher PIRG values in all isolates. The greatest inhibition was observed at 0.75 and 1.0 mg mL^{-1} , reaching complete suppression of radial growth in most isolates. Within each isolate, PIRG differed significantly among CuO-NP concentrations (Dunn–Holm, $p \leq 0.05$), indicating that higher CuO-NP levels enhanced antifungal activity.

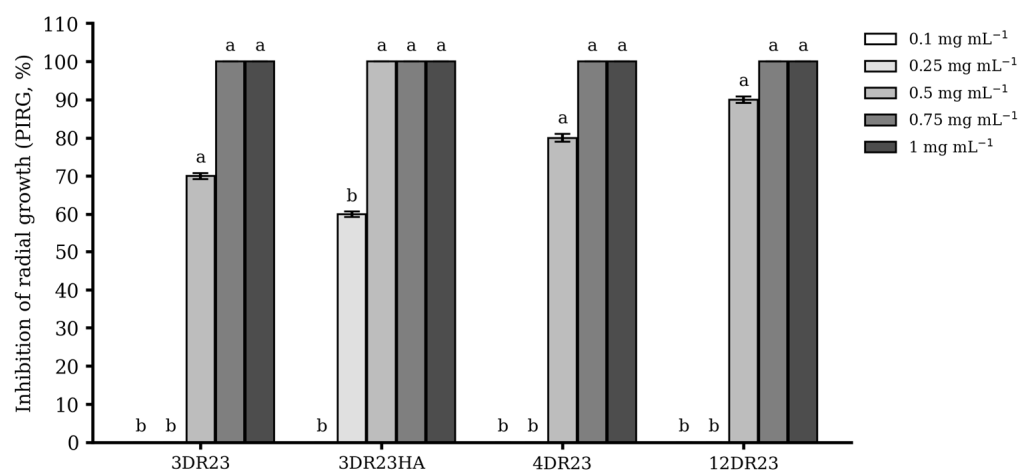


Figure 7. In vitro inhibition of radial growth (PIRG, %) by CuO-NPs against four *Fusarium* spp. isolates (3DR23, 3DR23HA, 4DR23, and 12DR23). Within each isolate, different lowercase letters indicate significant differences among CuO-NPs concentrations based on Kruskal–Wallis followed by Dunn’s multiple comparisons with Holm adjustment ($p \leq 0.05$) $\pm$ Standard deviation.

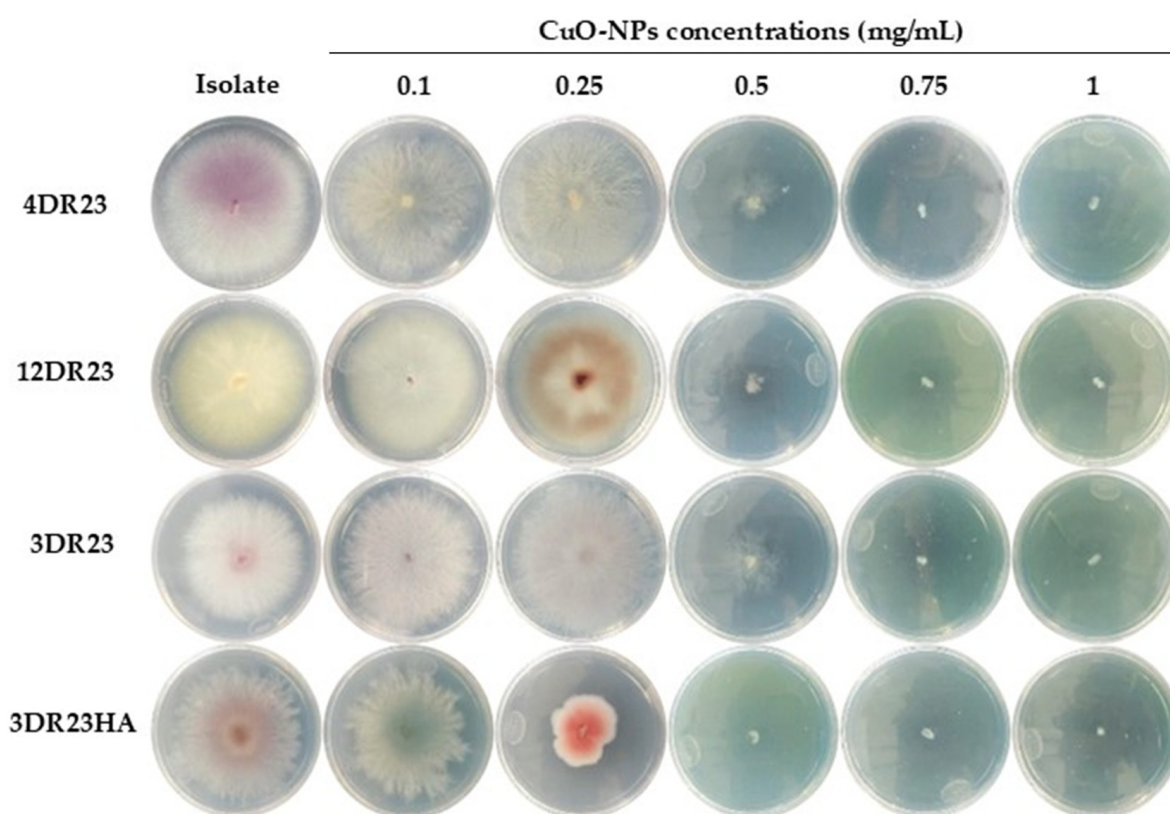


Figure 8. In vitro inhibition of *Fusarium* spp. by CuO-NPs. Isolates of phytopathogenic fungi: 4DR23, 12DR23, 3DR23, and 3DR23HA.

3.5. Effects of Integrated Treatments on Tomato Seedling Growth Under Phytopathogen Challenge

Table 2 shows that *F. oxysporum* (3DR23HA) reduced seedling growth, resulting in the lowest stem length (15.9 cm) and the lowest number of leaves (5.4 leaves plant⁻¹). In contrast, the synthetic fungicide, the bacterial strains (KN1 and KN2), and CuO-NPs applied individually generally improved growth relative to the phytopathogen-only treatment, with stem length values ranging from 24.1 to 26.9 cm ($p \leq 0.05$).

Table 2. Effects of *Stenotrophomonas rhizophila* (KN1, KN2), CuO-NPs, and their combinations on the growth of tomato seedlings infected with *Fusarium oxysporum* (3DR23HA).

Treatment	Stem Length (cm)	Stem Diameter (mm)	Root Length (cm)	Leaves (No./Plant)
Inoculated control (3DR23HA)	15.9 ± 1.7 ^{b†}	2.2 ± 0.4 ^a	9.6 ± 1.1 ^a	5.4 ± 0.9 ^a
Synthetic fungicide	25.8 ± 2.4 ^a	3.6 ± 0.5 ^b	13.4 ± 2.1 ^{ab}	7.0 ± 0.0 ^{ab}
KN1	24.1 ± 1.7 ^a	3.4 ± 0.5 ^b	16.4 ± 1.1 ^b	7.4 ± 0.9 ^b
KN2	25.3 ± 1.5 ^a	3.2 ± 0.4 ^{ab}	13.5 ± 2.2 ^{ab}	5.8 ± 0.4 ^{ab}
CuO-NPs 0.50	26.9 ± 3.1 ^a	3.2 ± 0.4 ^{ab}	18.4 ± 1.5 ^b	6.8 ± 0.4 ^{ab}
CuO-NPs 0.75	24.5 ± 1.1 ^a	3.0 ± 0.0 ^{ab}	14.5 ± 0.4 ^{ab}	7.0 ± 0.0 ^{ab}
KN1 + CuO-NPs 0.50	17.0 ± 1.7 ^b	3.2 ± 0.4 ^{ab}	11.2 ± 0.9 ^a	6.0 ± 0.7 ^{ab}
KN1 + CuO-NPs 0.75	24.8 ± 4.1 ^a	3.2 ± 0.4 ^{ab}	13.6 ± 1.1 ^{ab}	6.2 ± 0.4 ^{ab}
KN2 + CuO-NPs 0.50	16.8 ± 2.0 ^b	3.2 ± 0.4 ^{ab}	12.9 ± 3.5 ^{ab}	6.6 ± 0.9 ^{ab}
KN2 + CuO-NPs 0.75	23.1 ± 2.5 ^a	3.0 ± 0.0 ^{ab}	15.7 ± 3.6 ^{ab}	7.0 ± 0.0 ^{ab}

[†] Different lowercase letters within a column indicate significant differences among treatments ($p \leq 0.05$; Dunn's test with Holm adjustment). Factorial effects (bacteria, CuO-NPs, and A × B) were tested using the Scheirer–Ray–Hare two-way ANOVA on ranks; the synthetic fungicide was treated as a non-factorial reference and compared separately ± Standard deviation.

Stem diameter also increased relative to 3DR23HA, particularly in the synthetic fungicide and KN1 treatments (3.4–3.6 mm), whereas the phytopathogen-only treatment showed the greatest reduction (2.2 mm) ($p \leq 0.05$). Root length showed a positive numerical response to some individual treatments; however, statistically significant increases relative to the phytopathogen-only treatment were observed only for CuO-NPs at 0.50 mg mL^{−1} (18.4 cm) and KN1 (16.4 cm), compared with 9.6 cm in the phytopathogen-only treatment ($p \leq 0.05$).

Among the combined treatments, KN1 + CuO-NPs 0.75 and KN2 + CuO-NPs 0.75 maintained stem length values statistically comparable to those of the best-performing individual treatments and the synthetic fungicide, whereas KN1 + CuO-NPs 0.50 and KN2 + CuO-NPs 0.50 produced shorter stems (16.8–17.0 cm), similar to the phytopathogen-only treatment ($p \leq 0.05$). Overall, these results indicate that KN1 and CuO-NPs, particularly at 0.50–0.75 mg mL^{−1}, can mitigate the growth suppression caused by 3DR23HA, although the magnitude of the response depends on the specific bacterium–nanoparticle combination.

Figure 9 shows that inoculation with *Fusarium oxysporum* (3DR23HA) resulted in the lowest mean values for both fresh root biomass and fresh shoot biomass among the treatments evaluated. For fresh root biomass (Figure 9A), statistically significant increases relative to the phytopathogen-only treatment were observed for the synthetic fungicide, CuO-NPs at 0.50 mg mL^{−1}, KN1 + CuO-NPs 0.50 mg mL^{−1}, and KN1 + CuO-NPs 0.75 mg mL^{−1} ($p \leq 0.05$). The remaining treatments did not differ significantly from 3DR23HA. For fresh shoot biomass (Figure 9B), statistically significant increases relative to the phytopathogen-only treatment were detected only for the synthetic fungicide, KN1 + CuO-NPs 0.75 mg mL^{−1}, and KN2 + CuO-NPs 0.75 mg mL^{−1} ($p \leq 0.05$), with KN2 + CuO-NPs 0.75 mg mL^{−1} showing the highest mean value. Overall, treatments involving *S. rhizophila* and CuO-NPs mitigated the biomass reduction caused by *F. oxysporum*, although the magnitude of the response varied according to the variable evaluated and the treatment applied.

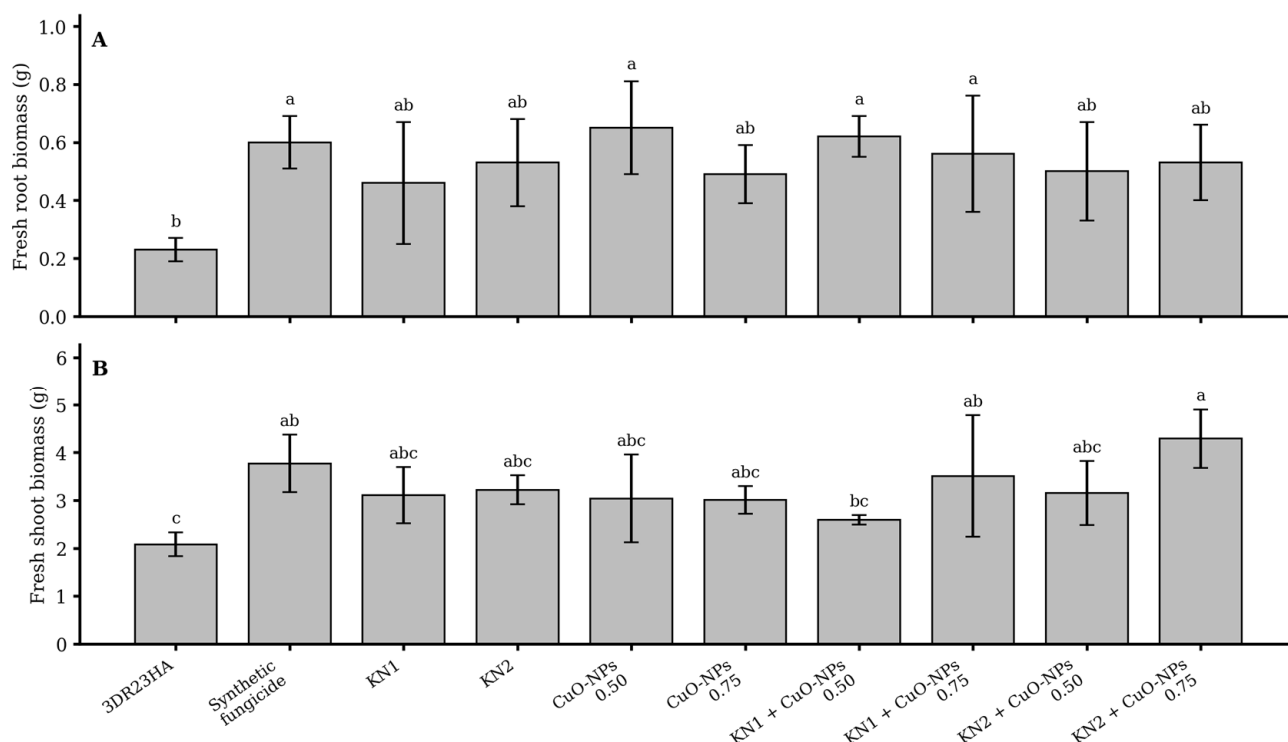


Figure 9. Effects of *Stenotrophomonas rhizophila* (KN1, KN2), CuO-NPs, and their combinations on the biomass of tomato seedlings infected with *Fusarium oxysporum* (3DR23HA). **(A)** Fresh root biomass (g) and **(B)** fresh shoot biomass (g). Different lowercase letters above bars indicate significant differences among treatments (Dunn's test with Holm adjustment, $p \leq 0.05$) $\pm$ Standard deviation.

3.6. Effects of Integrated Treatments on Disease Incidence, Severity, and Control Efficiency

In the phytopathogen-only control (3DR23HA), damping-off incidence was 100% and severity reached a DSI of 85.0%. Most treatments reduced incidence to 0–40% (relative reductions of 60–100%), and the KN1, KN1 + CuO-NPs 0.75, and KN2 + CuO-NPs 0.75 treatments achieved 0% incidence. However, no significant differences in incidence were detected among treatments ($p > 0.05$) (Figure 10A).

In contrast, severity (DSI, %) showed a significant treatment effect ($p = 0.00016$). The most effective treatments—KN1, KN1 + CuO-NPs 0.75, and KN2 + CuO-NPs 0.75—reached DSI = 0%, corresponding to a 100% relative reduction compared with the phytopathogen-only control (3DR23HA) and the synthetic fungicide. The KN1 + CuO-NPs 0.50 combination increased severity relative to KN1 alone (DSI = $40.0 \pm 13.7\%$, $\approx 52.9\%$ reduction vs. the control), suggesting an antagonistic effect at that dose/mixture, whereas at 0.75 the combination maintained complete control (DSI = 0%) (Figure 10B).

Control efficiency (Abbott, %) varied significantly among treatments ($p = 0.0071$). The highest efficiencies were observed for KN1, KN1 + CuO-NPs 0.75, and KN2 + CuO-NPs 0.75, all of which reached 100.0%. In contrast, the KN1 + CuO-NPs 0.50 combination showed the lowest efficiency (52.9%) and was significantly lower than KN1, KN1 + CuO-NPs 0.75, and KN2 + CuO-NPs 0.75 (Dunn–Holm, $p = 0.0092$). In terms of control efficiency (Abbott, %), none of the evaluated treatments differed significantly from the synthetic fungicide (Dunn–Holm, $p > 0.05$). Notably, several alternatives achieved maximum efficiency (100%), indicating performance comparable to the chemical treatment and supporting their potential as substitutes or complements within integrated damping-off management (Figure 11).

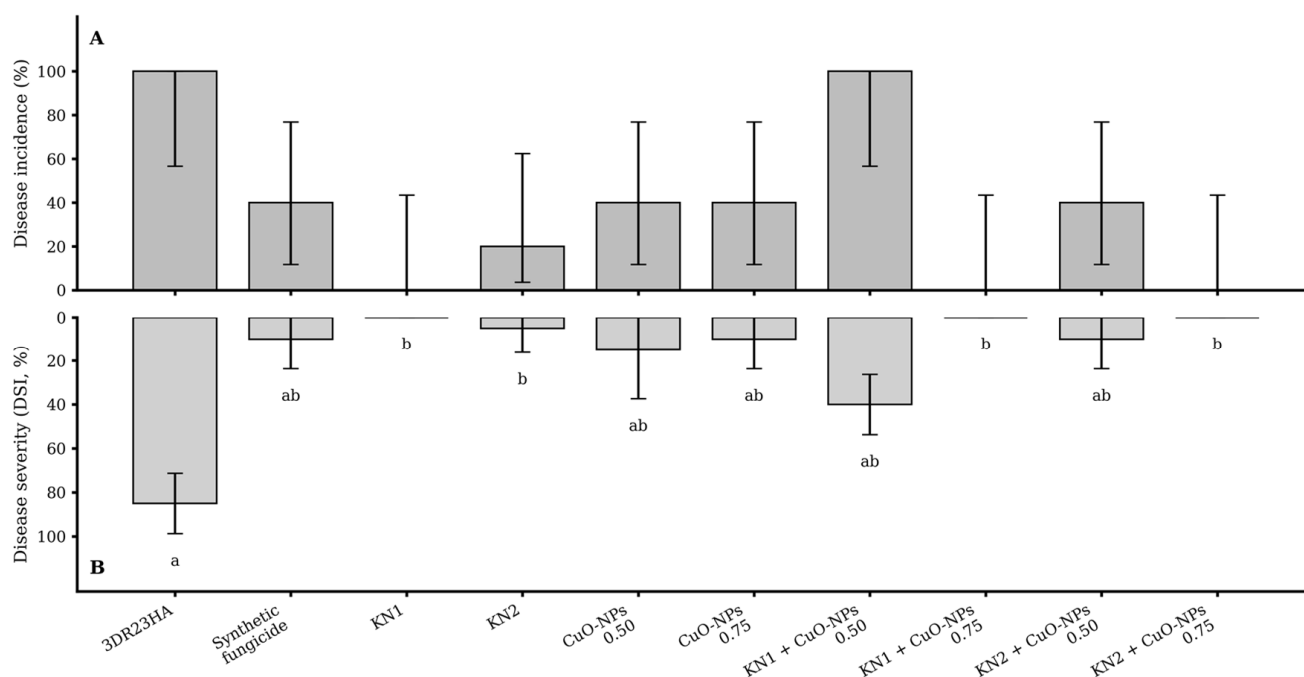


Figure 10. Disease incidence and disease severity (DSI, %) of tomato damping-off under integrated treatments with *Stenotrophomonas rhizophila* (KN1, KN2) and CuO-NPs. **(A)** Disease incidence (%) with 95% Wilson confidence intervals. **(B)** Disease severity expressed as disease severity index (DSI, %) (mean $\pm$ standard deviation). Severity bars are plotted downward for visual comparison with incidence. In panel B, different lowercase letters indicate significant differences in disease severity among treatments based on the Scheirer-Ray-Hare factorial test, followed by simple-effects comparisons and Dunn's test with Holm adjustment ($p \leq 0.05$). Treatments without letters did not show significant pairwise differences in the post hoc comparisons.

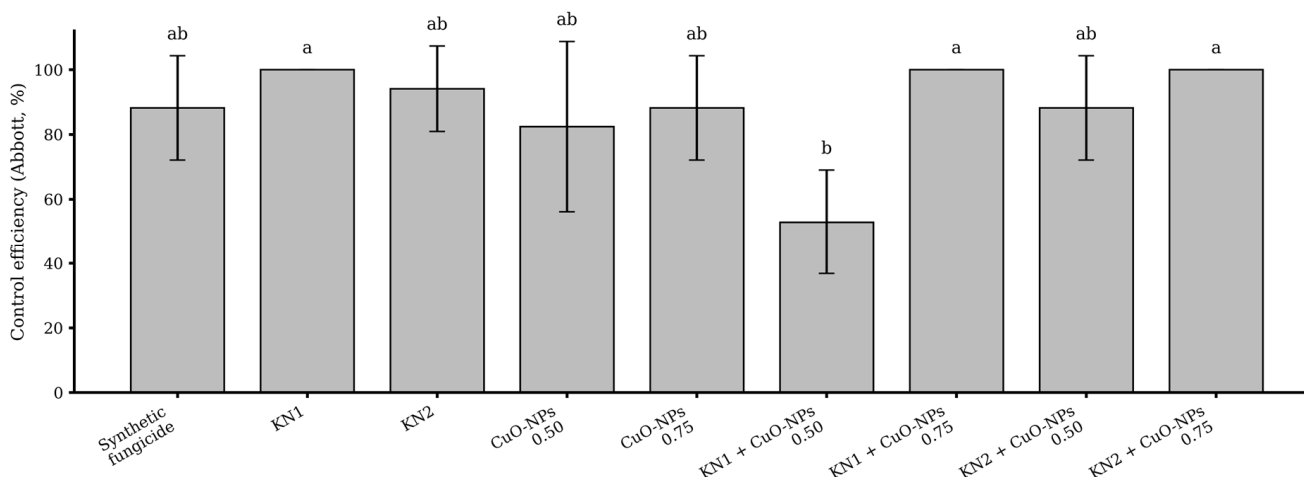


Figure 11. Control efficiency (Abbott, %) of integrated treatments based on disease severity in tomato damping-off. Different lowercase letters indicate significant differences among treatments (Dunn's test with Holm adjustment; $p \leq 0.05$) $\pm$ Standard deviation.

4. Discussion

Fusarium spp. are widely distributed in tomato production systems worldwide and represent a phylogenetically diverse group of fungi frequently associated with damping-off [59,60]. In the present study, the macroscopic and microscopic features of the isolates were consistent with the taxonomic descriptions of *Fusarium* spp. provided by Summerell et al. [46] and Nelson et al. [61]. The colony traits observed, including cottony aerial mycelium and variable pigmentation ranging from whitish to pink, violet, or

brown, agree with previous descriptions for this genus [62]. Likewise, the morphological variation recorded among isolates is consistent with the phenotypic plasticity reported for *Fusarium* spp., particularly with respect to colony pigmentation and structure [63,64].

The differential incidence and severity caused by the isolates are also biologically plausible, since aggressiveness in *Fusarium* spp. may vary according to isolate genetics, host susceptibility, and environmental conditions [65,66]. After conidia adhere to and invade plant roots, fungal colonization of the vascular system can disrupt water transport through the xylem, thereby promoting wilting, growth restriction, and, in severe cases, plant death [67]. Disease symptoms are associated with vascular blockage caused by fungal hyphae and by host–pathogen interaction processes, including the accumulation of toxins, gels, and tyloses, which ultimately lead to epinasty, chlorosis, wilting, defoliation, and death of the host plant [68,69]. At the early stages of disease development, growth and biomass reduction are expected because vascular dysfunction restricts the movement of water and nutrients through the xylem [70,71].

Our results support this pathophysiological framework. Isolate 3DR23HA showed the highest incidence (60%) and was associated with the greatest reductions in root biomass. Although severity clustered at the same ordinal level (grade 3 for most isolates), biomass, particularly root dry weight, provided greater sensitivity for discriminating damage intensity. This is consistent with damping-off pathosystems, in which external symptoms may appear similar among isolates, whereas the functional deterioration of the root system and the associated limitation in water and nutrient uptake may differ substantially [72,73].

In vitro confrontation assays are widely used as an initial screening step to identify microorganisms with potential for the biological control of phytopathogens [74]. In the present study, both *S. rhizophila* strains inhibited *Fusarium* spp., although the magnitude of antagonism depended on both the bacterial strain and the fungal isolate. Hernández-Montiel et al. [75] likewise reported strong inhibition of the mycelial growth of several phytopathogenic fungi, including *Colletotrichum gloeosporioides*, *Penicillium italicum*, *P. digitatum*, *Alternaria solani*, *Fusarium oxysporum*, *Neoscytalidium dimidiatum*, *Alternaria alternata*, *F. solani*, and *Curvularia* sp., by marine strains of *S. rhizophila*, with inhibition values ranging from 90 to 98% and from 88 to 97%, respectively, thereby supporting the high antifungal potential of this bacterial species. In our study, both *S. rhizophila* strains inhibited radial growth, but the magnitude of inhibition depended on the fungal isolate. KN1 inhibited radial growth by 63–79% across the four isolates, whereas KN2 produced inhibition values of 63–74% in three isolates but only 22% against isolate 12DR23. KN1 also showed greater inhibition of conidial germination than KN2. This distinction is epidemiologically relevant because conidial germination and early establishment are critical stages in disease initiation; therefore, an antagonist that interferes with these early events may reduce effective inoculum pressure even when subsequent mycelial expansion is not proportionally reduced [76,77].

The antagonistic activity observed for *S. rhizophila* may be related to mechanisms previously reported for this species, including hydrolytic enzymes, antibiosis, competition for nutrients and space, siderophore production, surfactant-associated effects, and volatile organic compounds [78–80]. From a microbiological standpoint, hydrolytic enzymes such as chitinases, glucanases, and proteases are of particular interest because they may act directly on structural components of the fungal cell wall, including chitin, β -glucans, and wall-associated proteins. The hydrolysis of these polymers may weaken cell wall integrity, compromise hyphal stability, and generate smaller oligosaccharides that can subsequently be assimilated by bacteria as carbon sources [81]. Although these mechanisms were not directly measured in the present study, the stronger suppression of conidial germination by KN1 suggests that strain-dependent traits may influence

the earliest stages of phytopathogen establishment, which is particularly relevant in damping-off, where disease onset depends heavily on successful infection shortly after seedling emergence.

The response of *F. oxysporum* to CuO-NPs was clearly concentration dependent. At 0.1–0.25 mg mL^{−1}, inhibition was limited, whereas concentrations from 0.5 mg mL^{−1} onward markedly reduced radial growth, with complete suppression in most isolates at 0.75–1.0 mg mL^{−1}. This result indicates that the antifungal effect of CuO-NPs was dose-dependent and that a threshold concentration was required to achieve strong inhibition. At the lowest concentration, changes in mycelial pigmentation were observed, which may reflect fungal stress responses, including the production of protective pigments such as melanin and carotenoids [82]. Previous studies have associated the antifungal activity of CuO-NPs with copper ion release, membrane perturbation, oxidative stress, and damage to cellular macromolecules [83–85]. At the cellular level, copper ions released from CuO-NPs may alter membrane permeability and redox balance, thereby promoting oxidative damage to lipids, proteins, and nucleic acids. Once the antioxidant and repair capacity of the fungal cell is exceeded, these alterations may become incompatible with normal hyphal growth and viability [84,85]. Although those processes were not directly measured here, the concentration-dependent inhibition observed in our assays is consistent with progressive cellular stress that becomes increasingly damaging at higher nanoparticle concentrations.

The in vivo results further support the role of *S. rhizophila* in damping-off management. In the individual treatments, both bacterial strains improved growth relative to the phytopathogen-only control, suggesting that their effect extended beyond pathogen suppression. This interpretation is consistent with previous reports describing *S. rhizophila* as a multifunctional plant-associated bacterium with potential roles in growth promotion, nutrient mobilization, and enhanced seedling performance under biotic stress [86–88]. In our study, this broader effect was reflected in the recovery of seedling vigor under phytopathogen challenge, which is agronomically relevant because damping-off management should not only reduce visible symptoms but also preserve seedling quality, biomass accumulation, and subsequent establishment capacity.

The integrated treatments provide the most important biological and practical outcome of the study. Under challenge with the most virulent isolate, the combinations containing CuO-NPs at 0.75 mg mL^{−1} (KN1 + CuO-NPs 0.75 and KN2 + CuO-NPs 0.75) achieved complete control of severity (DSI = 0%) and maximum control efficiency (Abbott = 100%), with performance comparable to that of the synthetic fungicide. Although incidence was numerically reduced to 0–40%, those differences were not statistically significant, whereas severity clearly discriminated among treatments. This pattern is consistent with the lower discriminatory power of incidence as a binary variable, whereas DSI integrates symptom intensity and is therefore more sensitive for separating treatment effects under high disease pressure [89].

The superior performance of the 0.75 mg mL^{−1} combinations may reflect complementary effects between *S. rhizophila* and CuO-NPs operating at different stages of the host-phytopathogen interaction. A biologically consistent interpretation is that *S. rhizophila* reduced the probability of early inoculum establishment in the rhizosphere, whereas foliar-applied CuO-NPs enhanced host defensive capacity through mechanisms previously reported in the literature. In the case of *S. rhizophila*, suppression of early infection may involve antibiosis and the production of lytic enzymes such as chitinases, glucanases, and proteases, which can hydrolyze major structural components of the fungal cell wall, including chitin, β -glucans, and wall-associated proteins, thereby weakening hyphal integrity and restricting phytopathogen establishment [79–81]. This antagonistic

effect may be further reinforced by competition for nutrients and space in the rhizosphere, siderophore-mediated iron sequestration, and the action of diffusible and volatile metabolites, all of which may reduce the likelihood of successful colonization during the earliest stages of infection [14,78–81].

Copper-based nanoparticles have also shown disease-suppressive effects in tomato–*Fusarium* pathosystems. In tomato plants challenged with *Fusarium oxysporum* f. sp. *lycopersici*, chitosan-loaded CuO nanoparticles reduced wilt severity by 91.5% at 1 mg L^{−1} and by 96.0–98.0% at 25–50 mg L^{−1} after 75 days; the same study also reported increases in plant height, dry weight, flowering, photosynthetic pigments, and the activity of chitinase and peroxidase [39]. Likewise, in tomato plants affected by *Fusarium solani* root rot, CuO nanoparticles derived from *Zizyphus spina* leaf extract reduced disease severity from 80.5% in the infected control to 9.17 ± 2.89% at 250 mg L^{−1}, lowered disease incidence to 4.17 ± 3.80%, and improved seedling vigor, chlorophyll pigments, plant growth, and enzymatic activity [90].

In our study, the superior performance of the integrated treatments is consistent with the possibility that CuO-NPs contributed to disease suppression not only through direct antimicrobial activity, but also through host-mediated responses. Previous studies have associated copper-based nanoparticles with increased activity and/or expression of antioxidant enzymes such as SOD, APX, and GPX, which contribute to redox homeostasis and attenuation of infection-associated oxidative stress [42,43]. CuO-NPs have also been linked to activation of the phenylpropanoid pathway, reflected in increased PAL activity and, in some cases, PPO activity, which may promote the accumulation of phenolic compounds and flavonoids involved in chemical defense and reinforcement of structural barriers [42,44]. In addition, CuO-NPs have been associated with signaling-mediated resistance, including jasmonate (JA) modulation and activation of pathogenesis-related (PR) genes, responses consistent with SAR/ISR-like states and a priming effect that may enable the host to respond more rapidly and effectively to phytopathogen challenge [45,91]. Although these physiological and molecular responses were not measured in the present study, they provide a plausible literature-based framework for interpreting the superior performance of the integrated treatments observed here.

Overall, our results suggest that integrating *S. rhizophila* with CuO-NPs at 0.75 mg mL^{−1} may provide an effective strategy, with performance comparable to that of the synthetic fungicide, for managing tomato damping-off. However, validation under nursery and field conditions, together with further compatibility and safety assessments, remains necessary.

5. Conclusions

The present study demonstrated that both *Stenotrophomonas rhizophila* strains and CuO-NPs have strong potential for managing tomato damping-off associated with *Fusarium oxysporum*. In vitro, KN1 and KN2 inhibited mycelial growth of the evaluated isolates, whereas KN1 showed greater suppression of conidial germination. CuO-NPs exhibited a clear concentration-dependent antifungal effect, with complete inhibition of radial growth in most isolates at 0.75 and 1.0 mg mL^{−1}. In vivo, the phytopathogen reduced seedling growth and biomass, whereas individual applications of KN1, KN2, and CuO-NPs improved several growth traits relative to the phytopathogen-only treatment. The most effective integrated treatments were KN1 + CuO-NPs 0.75 mg mL^{−1} and KN2 + CuO-NPs 0.75 mg mL^{−1}, which achieved complete suppression of disease severity (DSI = 0%) and 100% control efficiency, with performance comparable to that of the synthetic fungicide. Overall, these findings indicate that the integration of *S. rhizophila* with CuO-NPs, particularly at 0.75 mg mL^{−1}, represents a promising strat-

egy for managing tomato damping-off, although validation under nursery and field conditions is still required before practical recommendation.

Author Contributions: Conceptualization, R.G.C.-C. and L.G.H.M.; methodology, R.G.C.-C., C.R.-M. and L.G.H.M.; software, J.J.R.-P., J.A.T.-R. and R.J.H.-F.; validation, R.G.C.-C., L.H.-A. and L.G.H.M.; formal analysis, R.J.H.-F. and J.A.T.-R.; investigation, L.G.H.M.; resources, J.J.R.-P., L.H.-A. and R.J.H.-F.; writing—original draft preparation, R.G.C.-C., C.R.-M. and L.G.H.M.; writing—review and editing, R.G.C.-C. and L.G.H.M.; supervision, R.G.C.-C.; project administration, R.G.C.-C. and L.G.H.M. All authors have read and agreed to the published version of the manuscript.

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