International Journal of Plant & Soil Science

Volume 38, Issue 2, Page 167-181, 2026; Article no.IJPSS.152241
ISSN: 2320-7035

Antibacterial Effect of Farm Compost Enriched with *Trichoderma virens* against *Ralstonia solanacearum*, the Causative Agent of Bacterial Wilt in Tomatoes

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Authors' contributions

This work was carried out in collaboration among all authors. Author KKL designed the study, performed the statistical analysis, Authors NAC and ADLNGE wrote the protocol. Author PAG managed the analyses of the study. Author HNR managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i25974

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/152241

Original Research Article

Received: 03/12/2025
Published: 18/02/2026

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Cite as: *Leonce, KRA Kobenan, N'GUESSAN Aya Carine, Pakora Alex Gilles, Amari Dadé Ler-N'ogn Georges Elisée, Coulibaly Anne-Edwige, and Hié Nanclan Réné. 2026. "Antibacterial Effect of Farm Compost Enriched With Trichoderma Virens Against Ralstonia Solanacearum, the Causative Agent of Bacterial Wilt in Tomatoes". International Journal of Plant & Soil Science 38 (2):167-81. https://doi.org/10.9734/ijpss/2026/v38i25974.*

Abstract

Tomato cultivation is economically important from Ivory Coast in Africa. However, it is exposed to various soil pathogens, among which bacterial wilt is one of the most feared. The present study aims to optimize tomato production by controlling *R. solanacearum* through the use of *Trichoderma virens* in farmyard compost as an ecological and sustainable solution. The work was organized into two main parts : an *in vitro* analysis of the effect of T. virens extract on the growth *of R. solanacearum* and the characterization of the impacts of T. virens-enriched compost on the development of Petomech tomato plants in the presence of *R. solanacearum* in a semi-controlled environment. *In vitro*, three concentrations (25, 50, and 100 mg/mL) of *T. virens* extract and two strains of *R. solanacearum* (RUN 1539 and 1822) were used. The methodology used was the diffusion test. In a semi-controlled environment, two spore concentrations of *T. virens* (10^6 and 10^8 spores/mL) with two volumes of solutions (300 and 400 mL) were introduced into the compost. Various composite proportions of 25%, 50%, and 75% of this amended compost were used in the culture medium for the tomato plants. The results showed that the crude extract of *T. virens* significantly inhibited the growth of strains 1539 and 1822 at a concentration of 100 mg/mL, with 1.08 mm and 2.2 mm, respectively. In the greenhouse, a significant reduction in wilting (WI) and colonization (CI) indices was observed with the use of compost enriched with 75% *T. virens* at a volume of 400 mL of inoculum. These treatments significantly improved the agronomic parameters of tomato plants. Treatments combining high concentration (C2 = 10^8 spores/mL) with compost proportions of 50% and 75% led to the best results, reducing the disease by 90%. This study showed that the results obtained demonstrated a dose-dependent inhibition of *R. solanacearum* by *T. virens*. Thus, the results demonstrate that the combination of *T. virens* and compost constitutes a promising strategy for biological control of bacterial wilt in tomato caused by *R. solanacearum*.

Keywords: Biological control; Trichoderma virens; Ralstonia solanacearum; enriched compost; Trichoderma virens extract.

1. Introduction

Vegetable crops occupy a significant place among crops of global importance. These crops help combat poverty and are significant sources of income for producer families (James, 2010 ; FAO, 2022). Tomatoes (*Solanum lycopersicum*) rank first among the most widely grown and consumed vegetables, globally, with annual production exceeding 180 million tons as of 2023, driven by demand in both fresh markets and processing industries (FAO, 2024). However, this crop faces various phytosanitary challenges, including the emergence of diseases caused by soil pathogens such as *Ralstonia solanacearum*, the agent responsible for bacterial wilt (Hayward, 1991). *Ralstonia solanacearum* was first observed in Ivory Coast in 1984, when it was detected on eggplant crops in Adiopodoumé. In Yamoussoukro, market gardeners refer to this disease as sudden death. It poses a serious threat to tomato cultivation, especially in regions where favorable ecological conditions facilitate its spread (the Center-West, South, etc.) (N'dodo et al., 2019 ; Cellier et al., 2023). In these areas, the damage can be severe, affecting up to 90% of contaminated crops (Hayward, 1991) and resulting in yield losses of up to 100% depending on the variety (Fondio et al., 2010). Given this restriction, it is imperative to find effective and healthy control methods. Biological control could be considered, as it has a much more targeted impact and minimal persistence in the environment (Amoatey and Acqual, 2010). One such method involves using natural antagonists to prevent or reduce the spread of the parasite. The fungus *Trichoderma virens* has proven effective in combating various crop pathogens (Mouria et al., 2015). In addition, the use of agricultural compost enriched with *Trichoderma virens* is an innovative and sustainable method of controlling soil diseases. Composting, a traditional method of treating biological waste, not only enriches soil fertility, but also helps to increase microbial diversity, thereby creating a hostile environment for pathogens (Bulluck et al., 2002). The incorporation of *Trichoderma virens* into compost could thus increase its effectiveness as a biological control agent against *Ralstonia solanacearum*. It is in this context that this study was conducted, with the overall objective of improving tomato cultivation by controlling *R. solanacearum* through the use of *Trichoderma virens* in farm compost. Specifically, the study aimed to :

- Evaluate *in vitro* the antibacterial activity of *Trichoderma virens* extracts on *Ralstonia solanacearum.*

- Determine the effect of compost enriched with *Trichoderma virens* on the growth of tomato plants in the presence of *Ralstonia solanacearum* under semi-controlled conditions.

2. Materials and Methods

2.1 Presentation of the Study Area

Félix Houphouët-Boigny University in Cocody is a higher education institution under the Ministry of Higher Education and Scientific Research (MESRS) of Ivory Coast. It is located in the city of Abidjan, more precisely in the commune of Cocody. Built on an area of approximately 205 hectares, it is located in the Cocody district to the east of the city of Abidjan. Its approximate geographical coordinates are 5°20′54″N(latitude) et 3◦59′11″W (longitude) with a sub-equatorial climate zone.

2.2 Materials

2.2.1 Plant Material

The plant material consisted of the Petomech tomato variety. This cultivar has a cycle of 65 to 70 days after transplanting, is susceptible to disease, and has an average weight of 80 g per fruit.

2.2.2 Fungal Material

The fungal material is a strain of *Trichoderma virens* isolated from tomato plants collected in the Songon market gardening area (Fig. 1) by Coulibaly et al., (2022). Two forms of the fungal strain were used.

2.2.3 Bacterial Strains

Two bacterial strains, RUN 1539 and RUN 1822, originating from Songon and Man respectively, were used (Table 2). These strains, belonging to phylotype I, were characterized by N'Guessan et al., (2013).

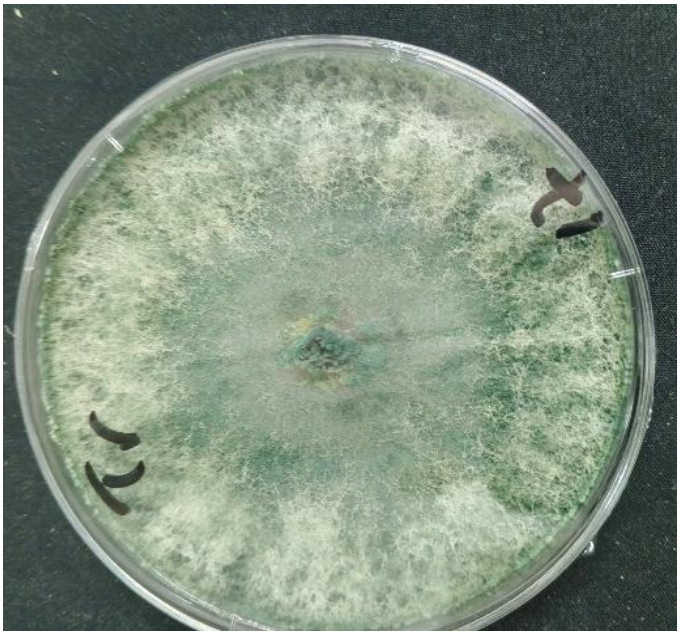

Fig. 1. *Trichoderma virens* with colonies aged 07 days on medium

Table 1. Isolation host, geographic origin, and phylogenetic positions

N°RUN	Code	Isolation host	Location (ZAE)	Phylotype
1539	CIV9	Tomato	Songon (I)	1
1822	CIV85	Eggplant	Man (III)	1

ZAE : (Agro-Ecological Zone)

2.2.4 Technical Equipment

The compost used was prepared from manure consisting of droppings and wood chip litter from free-range chicken farming. The soil used was sterilized before the experiment to eliminate any contamination.

2.3 Methods

2.3.1 *In vitro* Evaluation of the Antibacterial Activity of *Trichoderma virens* Extracts on *Ralstonia solanacearum*

The effect of *Trichoderma virens* extracts was tested at concentrations of 25, 50, and 100 mg/mL by diffusion on culture medium on the growth of *R. solanacerum*. To do this, an inoculum of each bacterial strain was prepared from 24-hour-old pure colonies. The bacterial suspension obtained was calibrated to an optical density of 0.2 at a wavelength of 600 nm, corresponding to 10^8 CFU/mL. One (1) mL of the prepared inoculum was distributed into Petri dishes containing CPG (Casamino, Peptone, Glucose) culture medium, then homogenized and the excess inoculum was removed. The dishes were left slightly open near a heat source for rapid drying for 3 minutes under a laminar flow hood. Then, three wells were made using a sterile 8 mm diameter punch. 40 µL of each concentration of *T. virens* extract was carefully deposited into the wells. A well containing sterilized distilled water was used as a reference. The study was repeated four times. The plates were pre-incubated at room temperature for one hour to allow pre-diffusion of the extract, then incubated in an oven for 24 hours at a temperature of 28°C. For each Petri dish, the diameters of the bacterial growth inhibition zones around the wells were measured.

2.3.2 Determination of the Effect of *Trichoderma virens* Enriched farm Compost on Tomato Plant Development in the Presence of *Ralstonia solanacearum* under Semi-Controlled Conditions

2.3.2.1 Preparation of Compost amended with *Trichoderma virens*

For the inoculation of *Trichoderma virens* strains into the compost, two (2) concentrations were prepared (10^6 and 10^8) spores/mL. According to the literature, concentrations of 10^6 and 10^8 represent the maximum inhibition concentrations. Using these spore concentrations of *T. virens*, two suspension volumes of 300 mL and 400 mL were prepared and then supplemented with 1.75 L of water and 250 mL of starch. These *T. virens* spore suspensions were inoculated into 50 kg of compost contained in a bin. The inoculated compost was covered with a black plastic sheet for incubation for one week at ambient conditions. After one week, three types of culture substrate were produced from the compost and sterile soil. These were mixtures (compost + soil) of 25%, 50%, and 75% compost inoculated with *T. virens*, which were compared with non-inoculated compost and sterilized soil.

2.3.2.2 Setting up the Nursery and Transplanting Tomato Seedlings

The nursery was set up in seed trays filled with a homogeneous mixture of unamended *Trichoderma* compost and sterilized soil. Seeds were sown in continuous rows spaced well apart to promote plant development. The nursery was then watered once a day, and the plants continued to grow for 21 days after sowing.

After obtaining the *Trichoderma virens*-amended compost, three types of tomato growing media were prepared by mixing it with sterile soil. The mixtures consisted of 25%, 50%, and 75% *Trichoderma virens* inoculated compost per 1000 g of growing medium (compost + soil). The 21-day-old tomato plants were transplanted into the different substrates. The plants were watered before inoculation.

2.3.3 Preparation of the Bacterial Inoculum and Inoculation of the Plants

The inoculum of *Ralstonia solanacearum* strains RUN 1539 and RUN 1822 was prepared as previously described. The plants were inoculated seven days after the application of *Trichoderma virens* to the substrate. For each pot, a volume of 5 mL of the bacterial suspension calibrated at 108 CFU/mL was administered to the plants at the collar. Ten (10) treatments were carried out depending on the type of substrate (Table 2).

2.3.4 Monitoring Disease Development under Semi-controlled Conditions

Symptoms were observed every three days over a period of 35 days after inoculation of tomato

plants with *R. solanacerum*. The evaluation was based on pathological and agronomic criteria. Tomato plants affected by bacterial wilt show distinctive signs such as yellowing, browning, and wilting of the leaves and stems. The pathological indicators were the wilting index (WI) and the colonization index (CI). The agronomic parameters were measured every seven days for 35 days. The agronomic parameters observed were the height, diameter, and number of living leaves on the plants.

- **Pathological parameters**

- **Wilting index (WI)** : This reflects the incidence of the disease. It was assessed using the Coupat-Goutaland et al. (2011) rating scale ranging from 0 to 4 :

The wilting index was calculated using the following formula :

$$WI = (N3 - N4) /NT \times 100$$

Where WI is the wilting index, N3 is the number of plants rated 3, N4 is the number of plants rated 4, and NT is the total number of plants observed.

- **The colonization index (CI)**

$$CI = IF + (NS \times RS)$$

Where NS is the percentage of asymptomatic plants ; RS is the percentage of asymptomatic plants with latent infection ; and IF is the wilting index.

Table 2. Inoculum volume and compost proportions for the different treatments for concentrations C1 (10^6 spores/mL) and C2 (10^8 spores/mL)

Treatment *Trichoderma virens*	Nams	Inoculum volume (mL) and concentration (spores/mL)	Proportion of compost (%)
T0sb	Control with bacteria-free compost	0	0
T01sb	Control with bacteria-free compost	0	25
T02sb	Control with bacteria-free compost	0	50
T03sb	Control with bacteria-free compost	0	75
T0b	Control with bacteria-free compost	0	0
T0b _25%	Sample with compost + bacter	0	25
T0b_50%	Sample with compost + bacter	0	50
T0b_75%	Sample with compost + bacter	0	75
Tr1_C1_25_%	Compost treatment	300 - (10^6)	25
Tr1_C1_50 _%	Compost treatment	300 - (10^6)	50
Tr1_C1_75_%	Compost treatment	300 - (10^6)	75
Tr2_C1_25_%	Compost treatment	400 - (10^6)	25
Tr2_C1_50 _%	Compost treatment	400 - (10^6)	50
Tr2_C1_75_%	Compost treatment	400 - (10^6)	75
Tr1_C2_25_%	Compost treatment	300 - (10^8)	25
Tr1_C2_50 _%	Compost treatment	300 - (10^8)	50
Tr1_C2_75_%	Compost treatment	300- (10^8)	75
Tr2_C2_25_%	Compost treatment	400- (10^8)	25
Tr2_C2_50 _%	Traitement avec compost	400 -(10^8)	50
Tr2_C2_75_%	Compost treatment	400- (10^8)	75

C1 = 10^6 spores/mL; C2 = 10^8 spores/mL; Tr1 = 300 mL volume of Trichoderma spore suspension ; Tr2 = 400 mL volume of Trichoderma spore suspension

Table 3. Rating scale

Numbers	Specific symptoms
0	0 No symptoms
1	1 One wilted leaf
2	2 Two or three wilted leaves or half of the leaves wilted
3	3 All leaves wilted
4	4 Stem bent or plant dead

At the end of the trial, latent infections were assessed on plants showing no symptoms.

In addition, strains were isolated in vitro from the stems in order to determine the possible presence of *R. solanacearum*. After disinfecting the stems, 2-3 cm sections were cut at the collar. These pieces were then placed in 5 mL of sterile distilled water and left for two hours at room temperature to allow the bacterial colonies to spread in the water (Prior et al., 1996). A volume of 50 µL of each suspension was spread on CPG medium using the three-sector method. The Petri dishes were then incubated at 28°C for 24 hours.

Asymptomatic plants are infected when colonies characteristic of *R. solanacaerum* are observed. The colonization index was calculated using data collected from latent infections (N'Guessan et al. 2013).

- **Agronomic parameters :** Plant height was measured from the collar to the terminal bud using a tape measure, while the diameter of the plant stem at the collar was measured using an electronic caliper. The living leaves of the plants were counted manually.

2.3.5. Statistical analysis of data

Data processing was performed using Excel spreadsheets. XLSTAT software version 2016.02.28451 was used for statistical analysis of the data. The variability of the colonization and wilting index was analyzed using this software. To this end, a normality test was performed, followed by Kruskal-Wallis tests and multiple pairwise comparisons, following Dunn's procedure (two-tailed test).

3. Results

3.1 *In vitro* Antibacterial Activity of *Trichoderma virens* Extracts on Bacterial Strains 1539 and 1822 of *Ralstonia solanacearum*

Fig. 3 shows areas of bacterial growth inhibition after application of *Trichoderma virens* extract at different concentrations. Fig. 3 illustrates the statistical study. This study reveals a notable difference between the various inhibition diameters. Nevertheless, the largest inhibition diameters were observed at a concentration of 100 mg/mL, with 1.08 mm and 2.2 mm for strains RUN 1539 and RUN 1822, respectively. No inhibition zone was noted around the control well containing sterile distilled water. However, the results showed an increasing sensitivity of *Ralstonia solanacearum* strains as the concentration of *Trichoderma virens* extract increased in the wells. Strain S2 (RUN 1822) showed the greatest sensitivity at all concentrations compared to strain RUN 1539.

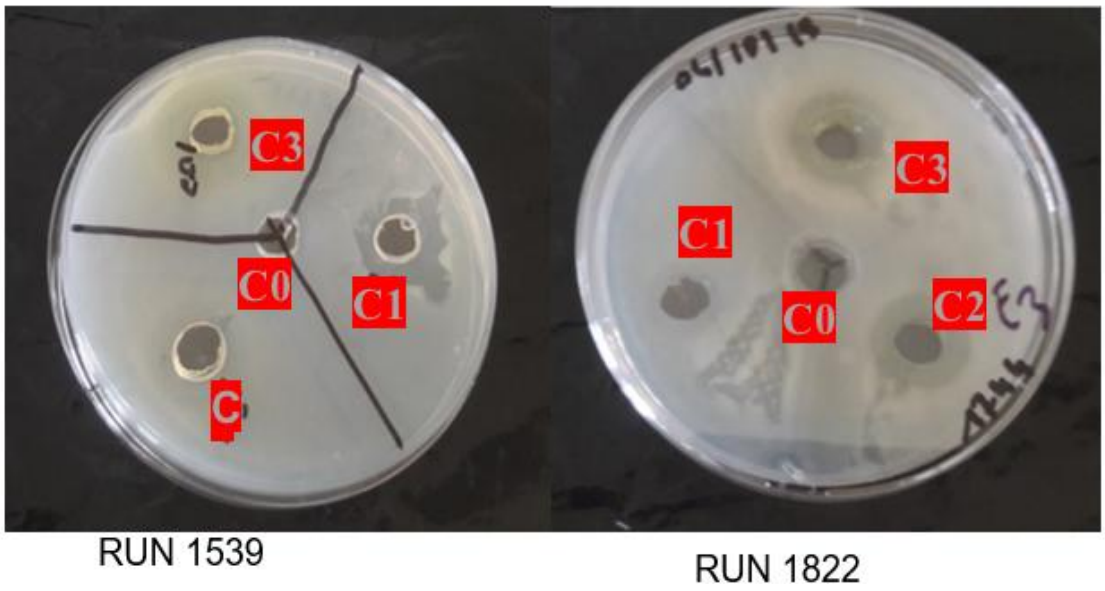

Fig. 2. Inhibition diameters of the bacterium *Ralstonia solanacearum* tested with different concentrations of *Trichoderma virens* extracts growing on CPG medium.

C0 ; C1 ; C2 ; C3 representing the concentrations of T. virens extract 0 ; 25 ; 50 and 100 mg/mL, respectively

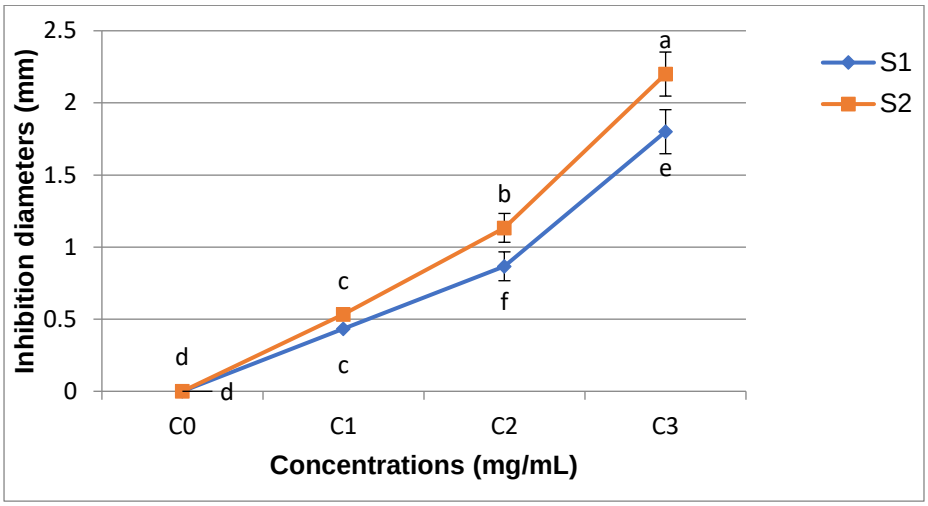

Fig. 3. Inhibition diameter of *Ralstonia solanacearum* strains at different concentrations of *Trichoderma virens* extract

C0 : Control with distilled water, C1 : concentration 25 mg/mL, C2 : concentration 50 mg/mL, C3 : concentration 100 mg/mL.

3.2 Effects of Compost Enriched with *Trichoderma virens* on the Growth of Tomato Plants infected with *R. solanacerum* in a Semi-controlled Environment

3.2.1 Effects on the Growth of Tomato Plants after Inoculation with Strain RUN 1539

The results in Table IV show that the growth parameters of tomato plants (collar diameter, number of living leaves, and plant height) vary significantly depending on the treatments applied ($p < 0.001$). The uninoculated control plants representing treatments T0sb, T0sb_25_%, T0sb_50_%, T0sb_75_% showed average diameters of 4.27 to 5.24 mm, numbers of living leaves of 5 to 5.7, and heights between 25.0 and 30.4 cm. These values reflect normal growth in the absence of the pathogen. In contrast, plants inoculated with R. solanacearum but not treated (T0b_%) showed a sharp reduction in growth, with diameters below 2.04 mm, numbers of leaves below 5.6, and heights not exceeding 22.3 cm, confirming the depressive impact of bacterial wilt on plant vigor.

The Kruskal-Wallis and Friedman tests confirmed a significant difference between the treatments applied at the two concentrations C1 and C2, with a significance threshold of 5%. Treatments at concentration C1 (Tr1_C1_25% to Tr2_C1_75%) significantly improved growth parameters compared to infected controls. Diameters of 2.99 to 4.49 mm, numbers of living leaves of 6 to 8, and heights of up to 40.6 cm show an average increase of 70 to 90%. However, the values remain generally lower than those associated with C2. Treatments at concentration C2 (Tr1_C2_50% to Tr2_C2_75%) produced an even more marked significant difference in all measured parameters. Diameters ranging from 4.59 to 5.50 mm, a number of leaves greater than 9, and heights up to 59.8 cm exceeded those of treatments C1. Treatment Tr2_C2_75% stood out with the highest values for all three parameters (5.50 mm; 9.38 leaves; 59.8 cm), indicating.

3.2.2 Effects on Tomato Plant Growth after Inoculation with Strain RUN 1822

Statistical analysis ($P < 0.001$) indicates that the treatments applied have a highly significant effect on the growth of tomato plants infected with strain RUN 1822.

The T0b treatments (bacteria alone) showed the lowest values for diameter (1.42-2.30 mm), number of leaves (5.8–8.68), and height(24.1–39.66 cm). These values reflect the plants' high sensitivity to the RUN 1822 strain, with marked growth inhibition and typical symptoms of bacterial wilt. In contrast, the T0sb treatments (bacteria-free compost) showed a notable

Table 4. Agronomic parameters of petomech variety tomato plants inoculated with the RUN 1539 strain of the bacterium *Ralstonia solanacearum* as a function of compost treatments and spore concentration. A synergistic effect between the high concentration of *T. virens* and the highest proportion of compost

Train 1539	Growth parameters of plants treated with Trichoderma concentrations C1 (10^6) and C2 (10^8)		
Treatments	**Diameters (mm)**	**Number of live leaves**	**Heights (cm)**
T0sb	4,27 ± 1,74 ab	5, 00 ± 3,02 de	25,00 ± 11,23 l
T0sb_25_%	4,83 ± 1,73 ab	5,40 ± 2,87 d	28,03 ± 1,32 k
T0sb_50_%	4,98 ± 1,06 ab	5,50 ± 2,87 d	28,29 ± 4,69 k
T0sb_75_%	5,24 ± 1,74 a	5,70 ± 2,49 d	30,45 ± 13,95 j
T0b_0 _%	1,39 ± 0,97 f	4,52 ± 2,69 e	16,15 ± 13,70 i
T0b_25_%	1,94 ± 1,27f	4,79 ±3,18 e	16,40 ± 13,64 i
T0b_50 _%	1,88 ± 1,33 f	5,50 ± 2,99 d	19,32 ± 11,23 h
T0b_75_%	2,04 ± 1,33 e	5,60 ± 1,39 d	22,32 ± 34,45 g
Tr1_C1_25_%	2,99 ± 1,23 e	6,35 ± 1,60 c	30,24 ± 13,61 f
Tr1_C1_50 _%	3,05± 1,27 d	6,75 ± 2,69 c	35,55 ± 14,93 f
Tr1_C1_75_%	3,28± 0,804 c	6,80± 0,91c	35,69 ± 14,62 e
Tr2_C1_25_%	3,24 ± 1,80 c	6,00 ± 4,00 bd	35,62 ± 16,94 e
Tr2_C1_50 _%	3,40 ± 1,59 c	6,95 ± 4,74 bd	40,52 ± 14,56 d
Tr2_C1_75_%	4,49 ± 1,32 ab	8,20 ± 4,24 b	40,63 ±14,55 d
Tr1_C2_25_%	3,452± 1,98 ab	8,18 ±4,64 b	41,72 ±14,04 d
Tr1_C2_50 _%	4,59 ± 1,68 ab	9,06 ± 4,01 a	48,30 ±12,53 c
Tr1_C2_75_%	4,69 ± 1,58 aab	9,62 ± 4,72 a	47,10 ±13,80 c
Tr2_C2_25_%	4,32 ±1,32 ab	6,56 ±4,39 c	49,40 ± 16,39 c
Tr2_C2_50 _%	5,48 ± 1,37 a	9,00 ± 4,29 a	52,40 ± 15,49 b
Tr2_C2_75_%	5, 50 ±1,19 a	9,38 ± 4,84 a	59,80 ± 0,99 a
P <0,001			

The means followed by different letters are significantly different at the 5% threshold according to the Kruskal-Wallis test and Dunn's multiple pairwise comparisons.

improvement in diameter, number of leaves, and height, ranging from 4.27 to 5.24 mm, 7.80 to 9.00, and 34.0 to 48.39 cm, respectively. However, treatments at concentration C1 (Tr1_C1 and Tr2_C1) showed moderate improvement in plant growth. Diameters, number of leaves, and heights ranged from 3.57 to 5.43 mm, 8.05 to 10.20, and 28.00 to 47.28 cm, respectively. However, the Tr2_C1_75% treatment was the most effective at this level, with a diameter of 5.43 mm, 10.2 leaves, and a height of 47.28 cm, indicating partial protection. Next, the Tr1_C2 and Tr2_C2 treatments (high concentration) showed a very significant improvement in diameter, with 5.07 and 6.18 mm respectively, in number of leaves, with 13.6 and 17.8 respectively, and in height, with 46.28 and 56.45 cm respectively. Finally, the Tr2_C2_75% treatment recorded the best overall results, with a diameter of 6.18 mm, a number of leaves of 17.81, and a height of 56.45 cm, demonstrating maximum biocontrol efficacy.

Finally, the Tr2_C2_75% treatment recorded the best overall results, with a diameter of 6.18 mm, a number of leaves of 17.81, and a height of 56.45 cm, demonstrating maximum biocontrol efficacy.

Table 5 shows the scale of treatment efficacy based on agronomic parameters. The Tr_C treatments showed significantly better growth than the infected controls.

The Tr2_C2_75% treatment, particularly at the 75% rate, showed the highest efficacy in reducing the effects of bacterial wilt caused by the RUN 1822 strain and in stimulating the growth of tomato plants.

3.3 Effect of *Trichoderma virens* on the wilting Index of plants Infected with *R. solanacerum*

3.3.1 Wilting Index of Plants after Inoculation with Strain RUN 1539

The mean wilting rate of tomato plants varied sigificantly according to spore concentration (C1 = 10^6 spores/mL; C2 = 10^8 spores/mL), inoculum

volume (300 or 400 mL), and compost proportion (25, 50, and 75%) (Figure 4). Statistical analysis revealed a highly significant difference ($p < 0.01$) between antagonist-treated plants and untreated controls. The highest wilting rate among controls was observed in T0b_0% (90%). No significant difference ($p < 0.01$) was found in wilting rates among compost-treated controls. Among antagonist treatments, the 25% compost proportion recorded the highest wilting rates, peaking at 60% for Tr1_C1_25%. Conversely, the lowest rates occurred with 75% compost : 10% for Tr2_C2_75% and 20% for Tr1_C2_75%. Kruskal-Wallis and Friedman tests confirmed significant differences ($p < 0.05$) between C1 and C2 concentrations.

Fig. 5 shows the results obtained for plants transplanted onto a substrate treated with the antagonist *Trichoderma virens* and inoculated with pathogenic strain 1822. The treatments, including plants treated with *T. virens* (Tr1_C1_25% to Tr2_C2_75%) and untreated controls (T0b to T0b_75%), revealed significant differences in wilting indices depending on the proportions of compost (25%, 50% and 75%) and the volumes of spore solution applied (300 and 400 mL). At concentration C1, the highest wilting index values were recorded for treatments Tr1_C1_25% (25%, 300 mL) and Tr2_C1_25% (25%, 400 mL), with 60% and 50% respectively. Similarly, at concentration C2, treatments Tr1_C2_25% and Tr2_C2_25% had the highest indices, reaching 40% and 30%, respectively.

Furthermore, the wilting indices observed on substrates containing 75% compost were statistically equivalent, regardless of the concentration of *T. virens* spores (Figure 5). A general trend indicates that the lowest wilting indices are associated with substrates enriched with T. virens, suggesting increased efficacy of the antagonistic fungus in reducing wilting symptoms. In contrast, the control treatment T0b_0% had the highest wilting index, reaching 90%.

Table 5. Agronomic parameters of tomato plants of the Petomech variety inoculated with strain RUN 1822 of the bacterium *Ralstonia solanacearum*, depending on the proportions of compost in the growing medium and the concentration of *Trichoderma* spores

Train 1822	Growth parameters of plants treated with *Trichoderma* concentrations C1 (10^6) and C2 (10^8)		
Treatments	**Diameters (mm)**	**Number of live leaves**	**Heights (cm)**
T0_%	4,27 ± 1,03 d	7,80 ± 1,29 j	34,02 ± 1,23 e
T0sb_25_%	4,83 ± 1,13 d	8,00 ± 1,033 f	36,02 ± 1,54 e
T0sb_50_%	4,98 ± 1,01ab	8,20± 1,44 f	39,5± 13,64 d
T0sb_75_%	5,24 ± 0,81 b	9,00 ± 1,00 e	48,39± 1,70 c
T0b_0 _%	1,42 ± 0,66 g	5,8 ± 1,03 i	28,45 ± 1,11 f
T0b_25_%	1,84 ±1,08 f	6,88 ± 0,81 h	24,10 ± 1,95 f
T0b_50 _%	1,95 ± 1,54 f	7,60 ± 3,82 g	34,70 ± 1,57 e
T0b_75_%	2,30 ± 1,58 e	8,68 ±4,03 f	39,66 ± 1,06 d
Tr1_C1_25_%	3,57 ± 1,47 b	8,05 ± 3,98 f	33,10± 13,15 e
Tr1_C1_50 _%	4,67 ± 1,51 d	8,18± 4,07 f	34,24 ± 1,60 e
Tr1_C1_75_%	4,65 ±1,65 d	8,68 ± 4,37 f	35,25 ± 1,68 ab
Tr2_C1_25_%	4,85 ± 0,30 d	9,00 ± 0,66 e	28,00 ± 1,58 f
Tr2_C1_50 _%	5,17 ± 1,80 c	10,00 ± 0,81 d	35,85 ± 0,93 e
Tr2_C1_75_%	5,43 ± 107 bc	10,20 ± 0,66 d	47,28 ± 1,53 c
Tr1_C2_25_%	5,07± 1,19 b	13,60 ± 3,40 c	35,85 ± 1,58 e
Tr1_C2_50 _%	5,24 ± 0,20 b	13,80 ± 0,96 c	46,28 ± 0,93 b
Tr1_C2_75_%	5,94 ± 1,01 a	14,50 ± 1,08 bc	47,71 ± 1,53 b
Tr2_C2_25_%	5,23 ± 0,81 b	15,50 ± 2,71 b	50,07 ± 1,54 ab
Tr2_C2_50 _%	6,00 ± 0,66 a	16,60 ± 0,81 a	54, 86 ± 5,60 a
Tr2_C2_75_%	6,18 ± 0,81 a	17,81 ± 1,39 a	56,45 ± 4,02 a
P <0,001			

The means followed by different letters are significantly different at the 5% threshold according to the Kruskal-Wallis test and Dunn's multiple pairwise comparisons

Table 6. Table showing the comparative effect of treatments

Treatments	Diameter (mm)	Living leaves	Height (cm)	Overall effectiveness
T0b	1,4 – 2,3	5,8 – 8,7	24 – 40	Very low
T0sb	4,3 – 5,2	7,8 – 9,0	34 – 48	Low to medium
Tr1_C1 / Tr2_C1	3,5 – 5,4	8,0 – 10,2	28 – 47	Medium
Tr1_C2 / Tr2_C2	5,0 – 6,2	13,6 – 17,8	46 – 56	Very high
Tr2_C2_75%	6,18	17,81	56,45	Maximum

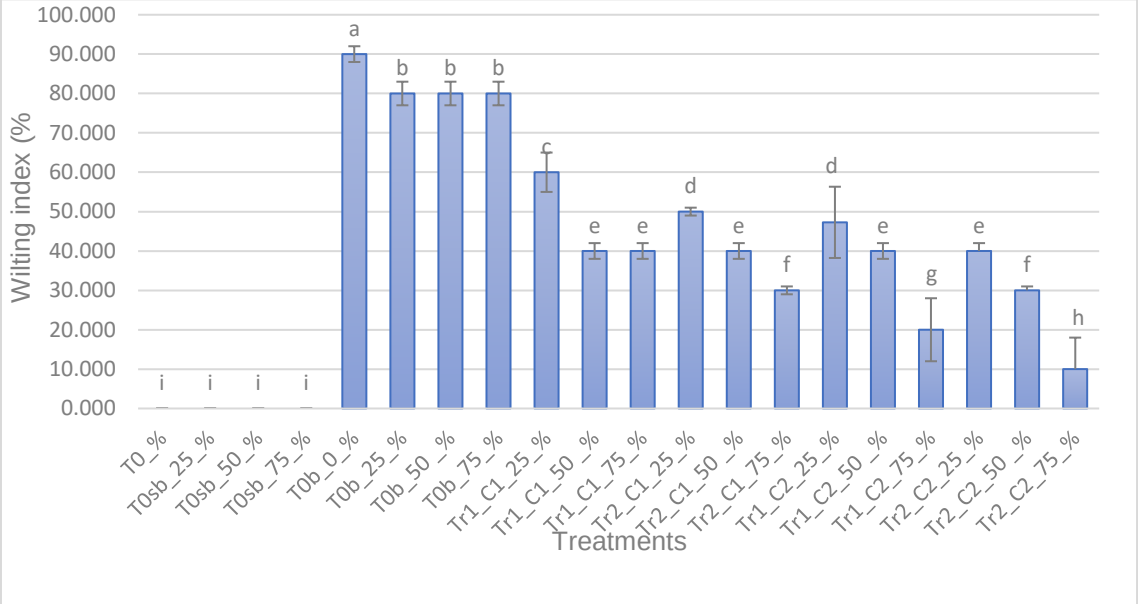

Fig. 4. Wilting index of Petomech plants inoculated with strain 1539 according to compost treatments at concentrations C1 [10⁶ spores/mL] and C2 [10⁸ spores/mL]

Bars with the same letters indicate that there are no significant differences between treatments at the 5% threshold according to the Kruskal-Wallis and Friedman tests

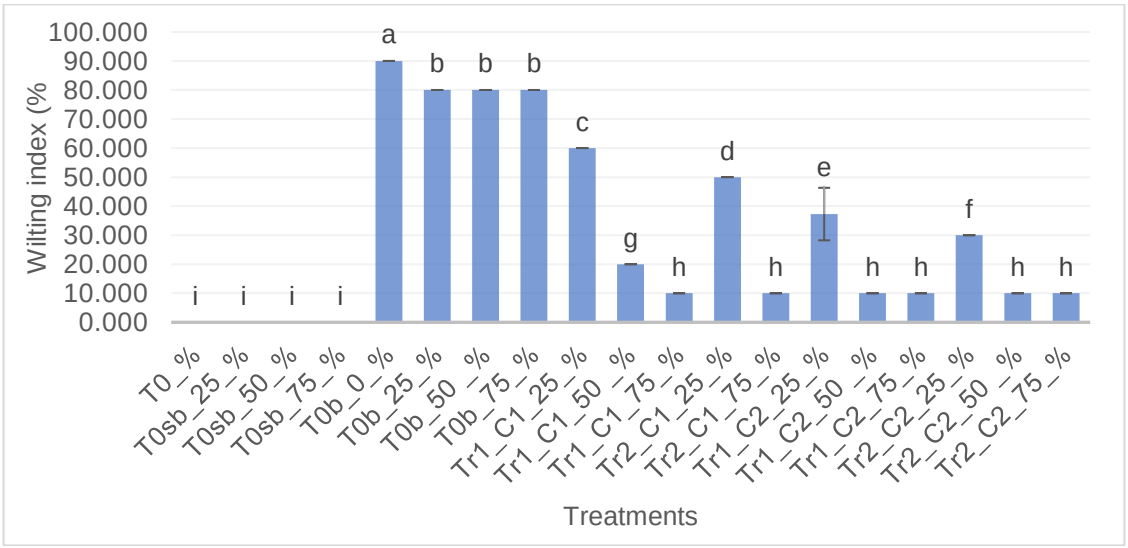

Fig. 5. Wilting index of Petomech plants inoculated with strain 1822 according to compost treatments at concentrations C1 [10⁶ spores/mL] and C2 [10⁸ spores/mL]

Bars with the same letters indicate that there are no significant differences between treatments at the 5% threshold according to the Kruskal-Wallis and Friedman tests

3.4 Effect of *Trichoderma virens* on the Colonisation Index of Plants

3.4.1 Colonisation Index of Plants Inoculated with Strain RUN 1539

The bacterium *R. solanacearum* was detected in the stems of tomato plants (*Solanum lycopersicum*) of the Petomech variety, which had been treated with the antagonist *Trichoderma virens* and then inoculated with the bacterial strain RUN 1539. Despite the detection of the bacterium, no symptoms of bacterial wilt were observed on these plants. The evaluation of bacterial colonisation according to compost proportions (25, 50 and 75%) and spore concentrations C1 (10^6 spores/mL) and C2 (10^8 spores/mL) revealed significant differences between the control plants inoculated with the bacterium and those treated with the antagonist. In the control treatments, colonisation indices were highest, reaching 90% for treatments T0b and T0b_25%, and 80% for T0b_50% and T0b_75%, with strain RUN 1539. In contrast, no significant differences were observed between treatments Tr1_C1_50%, Tr1_C1_75%, Tr1_C2_50%, Tr1_C2_75%, Tr2_C1_50% and Tr2_C2_75%, whose average colonisation rate was around 10% (Figure 6). A general trend indicates that the higher the spore concentration, inoculum volume and proportion of compost, the lower the bacterial colonisation rate. Thus, increasing the proportion of *T. virens* enriched compost in the substrate is accompanied by a significant reduction in *R. solanacearum* colonisation. For substrates containing 25% compost, the colonisation rates observed, all concentrations combined (C1 and C2), remain moderately low, with respective values of 19.8%, 30% and 42.2%.

3.4.2 Colonisation Index of Plants Inoculated with Strain RUN 1822

The results in Fig. 7 shows that untreated plants inoculated with the bacterium had significantly higher colonisation indices than those treated with the antagonist *Trichoderma virens* ($p < 0.05$). Thus, untreated plants (controls) and those treated with the antagonist recorded colonisation indices of 92.2% and 10%, respectively.

Furthermore, the Tr1_C1_25% and Tr1_C2_25% treatments had colonisation indices of 45.8% and 43.8% respectively. In contrast, the Tr2_C1_25% and Tr2_C2_25% treatments, with 33.8%,

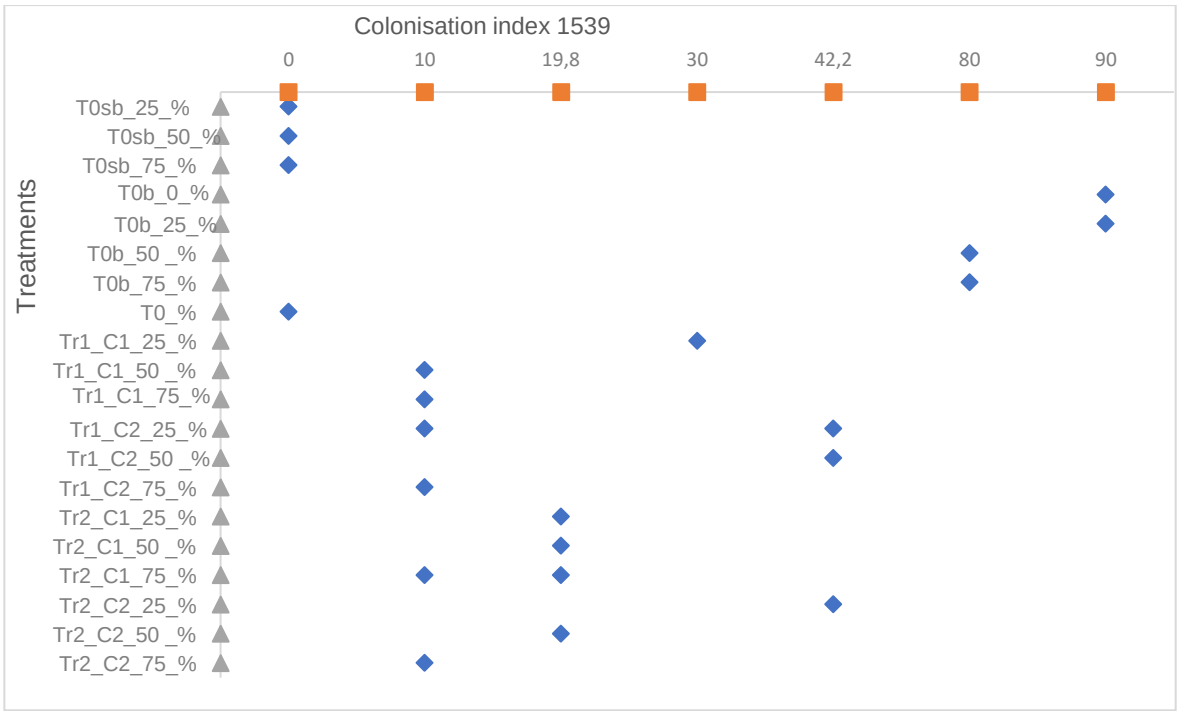

Fig. 6. Colonisation index of Petomech plants inoculated with strain 1539 according to compost treatments and concentrations C1 [10^6 spores/mL] and C2 [10^8 spores/mL]

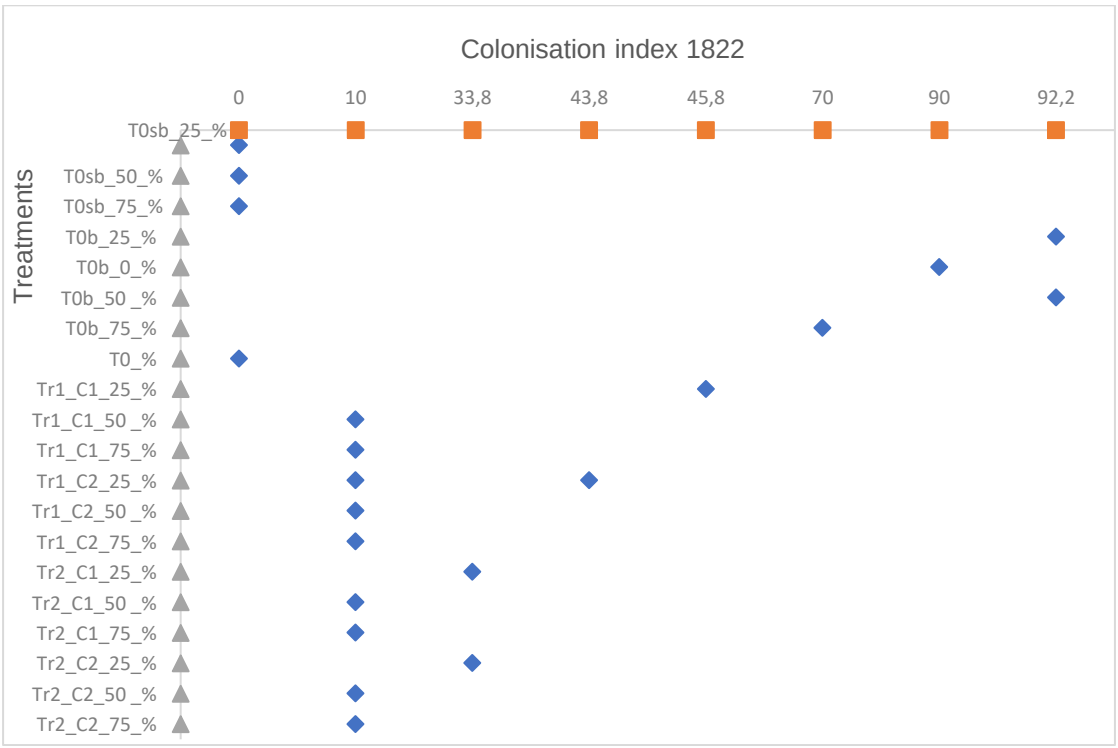

Fig. 7. Colonisation index of Petomech plants inoculated with strain 1822 according to compost treatments and concentrations C1 [10^6 spores/mL] and C2 [10^8 spores/mL]

showed significantly lower colonisation indices. However, the lowest colonisation levels were obtained with treatments where the proportion of compost was 50% and 75%, i.e. 10%, showing a marked reduction in bacterial colonisation under these conditions.

4. Discussion

The *in vitro* study revealed that strain S2 (1822) of the bacterium *Ralstonia solanacearum* is more sensitive than strain S1 (1539) to the antagonist *Trichoderma virens*. This sensitivity was observed with inhibition diameters of 1.80 and 2.2 mm induced by *Trichoderma virens* extract on strains RUN 1539 and 1822, respectively. This is attributed to the action of a set of secondary metabolites in the crude extract of *Trichoderma virens* on *Ralstonia solanacearum*, which causes bacterial wilt in tomatoes. These results corroborate those of Coulibaly et al., (2022), who point out that T*richoderma* produces antibiotics that are sometimes responsible for the inhibitions observed.

In the greenhouse, the enriched compost proved effective in proportions of 50% and 75% in treatments Tr1_C1_50%, Tr1_C1_75%, Tr1_C2_50%, Tr1_C2_75%, Tr2_C1_50% and Tr2_C2_75%. The results relating to the influence of the different treatments on the development and growth of tomato plants revealed higher diameters, heights and numbers of leaves in the treated plants of the Petomech variety. These growth parameters ranged from 1.42 to 6.18 mm in diameter, 24.10 to 56.45 cm in height and 5.80 to 17.81 leaves. These results are thought to be due to the presence of hydrolytic enzymes in the enriched compost, which cause the cell walls of *R. solanacearum* strains to break down. They are consistent with those of Benítez et al., (2004), who identified hydrolytic enzymes such as cellulases, chitinases and β-glucanases, which play an essential role in the decomposition of the cell walls of pathogens.

Furthermore, the generation of volatile organic compounds (VOCs), such as 6-pentyl-α-pyrone, mentioned by Contreras-Cornejo et al., (2014), has been recognised as a compound that inhibits the growth of pathogenic bacteria while activating the defence mechanisms of host plants and stimulating the expression of genes associated with induced systemic resistance (ISR). In this research, the dose-response correlation noted, where higher concentrations of *T. virens* spores increase antibacterial efficacy, is consistent with

the studies by Verma et al., (2007). These authors demonstrated that the cell concentration of *Trichoderma virens* is related to its competence in producing bioactive metabolites. The adaptive response capacity of T. virens gives it great flexibility as a biological control agent in various environmental contexts.

The use of *Trichoderma virens* in enriched compost significantly reduced the impact of *Ralstonia solanacearum* on pathological indicators (wilting and colonisation index). A significant reduction in pathological parameters was observed, particularly with a substantial volume (400 mL of *Trichoderma virens*) at a rate of 75% enriched compost. The compost used appears to limit the spread of *R solanacearum* in the soil. These results are consistent with those of Bulluck et al., (2002), who demonstrated that compost enriched with beneficial microorganisms has the ability to improve soil health by stimulating microbial biomass growth and enzymatic activity.

As for *Trichoderma virens*, it contributes to improving soil structure, optimises water retention and increases nutrient availability. This promotes plant development while preventing the spread of pathogens (Gravel et al., 2007). In this study, the synergistic effect observed between compost and *Trichoderma virens* could also be attributed to *Trichoderma virens'* ability to effectively colonise the rhizosphere and establish a beneficial interaction with the local microbiome. These results highlight the importance of an integrated approach that combines biocontrol agents with organic amendments to ensure sustainable crop protection. The RUN 1822 strain appears to have slightly lower virulence than the RUN 1539 strain, which may be attributed to genetic differences affecting their virulence. However, Monteiro et al., (2019) have demonstrated that *Trichoderma virens* has the ability to generate specific enzymes, such as DNases and proteases, which can break down these biofilms. This improves its effectiveness even against resistant strains. According to Harman et al., (2004), combining *Trichoderma virens* with other biocontrol agents, such as *Bacillus subtilis* or *Pseudomonas fluorescens*, could be a more economical and sustainable alternative. Singh et al., (2014) also suggested that integrating various biocontrol agents could strengthen the resilience of agricultural systems against emerging pathogens.

5. Conclusion

The aim of this study was to improve tomato production by combating *R. solanacearum* through the use of *Trichoderma virens* in farmyard compost. At the end of this study, the largest inhibition diameters induced by *Trichoderma virens* on strains RUN 1539 and 1822 were recorded at a concentration of 100 mL/L. Thus, *Trichoderma virens* extract inhibited the proliferation of *Ralstonia solanacearum* in vitro. A semi-controlled study of the antibacterial activity of *Trichoderma virens* revealed that a substrate consisting of 50% and 75% *Trichoderma virens* spore solution in a volume of 400 mL is effective against the bacterium *Ralstonia solanacearum*. The lowest wilting and colonisation indices, estimated at 10%, were observed at concentrations of 10^6 and 10^8 spores/mL, respectively. The treatments Tr1_C1_50%, Tr1_C1_75%, Tr1_C2_50%, Tr1_C2_75%, Tr2_C1_50% and Tr2_C2_75% resulted in good development of the tomato plants. However, further work is needed. Therefore, in view of this study, the treatments that proved effective in the greenhouse will need to be evaluated under field conditions.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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