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DEVELOPMENT OF A FORMULATION BASED ON EXTRACTS OF THE COCULTURE OF *Trichoderma* SPP *P3* AND *Streptomyces cameroonensis* TO FIGHT AGAINST *Ralstonia solanasearum* AN AGENT OF POTATO WILT DISEASE

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ABSTRACT

Potato is a crucial staple crop in Africa, particularly in Cameroon, but its production is hindered by pests and diseases. One of the most devastating of these is bacterial wilt, caused by the bacterium *Ralstonia solanacearum* and for which there is no known chemical control method. Bioformulations from microorganisms could potentially control this disease. This study tested the potential of a formulation based on an extract from a *Trichoderma* sp. *P3* and *Streptomyces cameroonensis* co-culture to control bacterial wilt. The compatibility of *Trichoderma* sp. *P3* and *Streptomyces cameroonensis* against phytopathogens, including *Ralstonia solanacearum,* was assessed. Two formulations based on extracts of *Trichoderma* sp. *P3* and of its coculture with *Streptomyces cameroonensis* were developed and their modes of action against *Ralstonia solanacearum* were explored. The results showed that *Trichoderma* sp. *P3* and *Streptomyces cameroonensis* can grow together in dual culture; the latter accelerates the maturation of the former, despite inhibiting its growth. Extracts of *Trichoderma* sp. *P3* and its coculture with *Streptomyces cameroonensis* exhibited different GC-MS profiles and greater inhibitory effects against phytopathogens than *Streptomyces cameroonensis* extract alone. The bioformulation developed using these two extracts had the same minimal inhibitory concentration, acting on *Ralstonia solanacearum* by hydrolysing its cell wall and reducing its ATPase-H^+ pumps. Pre-treatment of potato leaves reduced disease severity similar to chemical treatment. This study suggests that *Trichoderma* sp. *P3* and its coculture with *Streptomyces cameroonensis* have the same effect on pathogens, albeit via different modes of action. This could be further explored for controlling bacterial wilt.

Keywords: Bioformulation, Potato, *Ralstonia solanacearum, Streptomyces cameroonensis* and *Trichoderma* sp. *P3* consortium

INTRODUCTION

Potato (*Solanum tuberosum* L) is the fourth major crop produced globally after maize, rice, and wheat **(FAO, 2024)**. Potato is one of the strategic food crops in Africa for food and income security, its production has grown fast in Africa due to population growth, urbanization, and lifestyle changes **(Muthoni and Shimelis, 2023)**. In Cameroon, potato is cultivated mainly in the highland regions with altitudes ranging from 1000 to 3000m above sea level and in six of the ten regions, providing a source of food and income for many. Over 80% of national production comes from the West and North-West regions of Cameroon **(Lendzemo *et al.*, 2024).** During the last two decades' potato production increased almost threefold from 130535 tons in 2000 to 364284 tons in 2023 **(FAO, 2024),** this was driven largely by the ever-increasing domestic demand and a large export market to neighbouring countries like Gabon, Equatorial Guinea, Chad, Nigeria and Central Africa Republic **(Achiri *et al.*, 2018).** Despite increased production in Cameroon, the potato sector faces constant challenges of pests and diseases.

Bacterial wilt caused by the soil-borne bacterium, *Ralstonia solanacearum,* is among the most important and challenging diseases to manage in potato production in Cameroon. Infested soils and infected potato seed tubers and plants are the main sources of disease inoculum, but contaminated surface water used for irrigation and infested farm tools may also spread the disease**.** There is no efficient method to control bacterial wilt in potato as it can survive in soil and in the rooting system of many hosts including weeds **(Rado *et al.*, 2015).** Commonly recommended strategies to control the disease include crop rotation with non-host plants, rogueing of infected plants in the field, planting disease-resistant varieties, and the use of quality planting material. These methods alone cannot effectively control bacterial wilt. Lack of effective chemical control has also made it difficult to manage the disease. Integrated and sustainable disease management options are, therefore, needed to control this highly destructive and challenging disease.

The current biocontrol strategy employs the blending of various biological control agents (BCAs) of different microbial species having plant growth-promoting attributes in order to achieve desired outcomes **(Ram *et al.*, 2022).** Several studies have been done on the combined use of *Trichoderma* spp. and bacteria, mainly *Bacillus* spp. and *Pseudomonas* spp., for biocontrol or plant growth promotion on various crops **(Hafiz *et al.*, 2022; Izquierdo-Garcia *et al.*, 2020; Poveda and Eugui, 2022).** Strains belonging to the genera of *Trichoderma* and *Streptomycetes* have been widely used in biological control applications and reported as being effective against many phytopathogenic fungi **(Panchalingam *et al.*, 2022).** In Yaounde, Cameroon, some studies were carried out to identify some beneficial organisms for plant protection belonging to *Trichoderma* spp. and *Streptomyces* spp. **(Bedine Boat *et al.*, 2019; Boudjeko *et al.*, 2017).**

Trichoderma spp. have been widely studied, and the genus are filamentous fungi reported to be widely distributed in various ecosystems. This ubiquitous genus has a highly competitive nature for space and nutrients, reproductive ability, and capacity to promote plant growth and tolerate pesticides **(Bedine Boat *et al.*, 2019; Messi *et al.*, 2018; Silva *et al.*, 2012)**. *Trichoderma* isolates from Amazon Forest soil samples have shown potential for sheath blight (*Rhizoctonia solani*) suppression in rice and promote plant growth when applied as seed treatment, substrate incorporation, or foliar spray **(Silva *et al.*, 2012)**. Furthermore, seed treatment with *Trichoderma gamsii* isolated from Cameroon was reported to have a beneficial effect on the emergence of bean plants by inhibiting damping-off incidence and delaying the onset of disease symptoms induced by different plant pathogens, thus proposed as a promising alternative to control plant pathogens **(Bedine Boat *et al.*, 2019).** From soil samples collected in bean rhizosphere, **Bedine Boat *et al.* (2019)** isolated 85 *Trichoderma* spp among which the strain *Trichoderma* P3 and all these antagonists significantly inhibited ($P < 0.05$) the radial growth of the pathogens. *F. oxysporum, F. solani, M. phaseolina and P. ultimum.*

Among bacteria communities, actinobacteria have been reported to comprise several biocontrol agents that suppress plant diseases; they produce about 45% of the antibiotics currently in use, among which the genus *Streptomyces* alone produces 73% of the metabolites known to be capable of suppressing plant diseases. *Streptomyces cameroonensis*, an actinomycete isolated from the *Chromolaena odorata* rhizosphere in Yaoundé (Cameroon), was shown to exhibit

extensive antimicrobial effects against a wide range of microorganisms and traits of plant growth-promoting rhizobacteria (PGPR). Plant assays performed on cocoa plantlets confirmed that strain JJY4^T exhibited strong abilities to promote plant growth and protect against plant diseases **(Boudjeko *et al.*, 2017)**. The efficiency of a bioformulation of *Streptomyces cameroonensis* for the control of black pod disease in cocoa and enhancement of seedling growth was equally demonstrated by **Dzelamonyuy *et al.* (2022, 2023)**. According to several authors including **Viaene *et al.* (2016)** and **Ling *et al.* (2020),** *Streptomycetes* are well known to produce bioactive molecules with an antagonistic function against plant pathogens and are gaining interest in agriculture as plant growth promoting (PGP) bacteria and/or biological control agents (BCAs).

Two microbial strains with probably different modes of action were used in this study. The combination of beneficial fungi and bacteria offers great potential for microbe-assisted crop protection. Moreover, the combination of metabolites of these microorganisms has been demonstrated to have synergistic effect on plant pathogens **(Prigigallo *et al.*, 2023).** The central idea behind using microbial consortium is that a single microorganism may not necessarily give protection against a wide range of pathogens so using a group of micro-organisms will surely provide protection against multitargeted pathogens. Thus, the use of microbial consortium not only accelerates disease suppression but also give a positive impact on plant growth promotion **(Stockwell *et al.*, 2011; Bhatia *et al.*, 2018).** The expansion of the use of different microbial consortia, as well as an increase in research on different mixtures of microorganisms that facilitate the best and most consistent results in the field, was proposed by **Santoyo *et al.* (2021)**. The effect of consortium application of endophytic bacteria and fungi on plant growth, grain yield, and moisture status was conducted by **Muhae-Ud-Din *et al.* (2018)** and showed an enhancement of the various yield attributes of some wheat lines. Biological control using plant-beneficial microorganisms (or their metabolites), with their ability to improve crop productivity and resilience, could be an important alternative for the sustainable control of bacterial wilt in potato. However, the combination of *Trichoderma* spp. with *Streptomyces* spp. or their metabolites to control bacterial wilt in potato has been under explored.

The objective of the present study was to evaluate the potential of bioformulations developed from extracts of *Trichoderma* sp *P3* and of its coculture with *Streptomyces cameroonensis* to control potato wilt disease caused by *Ralstonia solanacearum.* We hypothesised that these extracts, either individually or in combination, could suppress the growth of *Ralstonia solanacearum* and be used as a sustainable, biological alternative to controlling the pathogen in potato production. To achieve that objective, the compatibility of the *Streptomyces cameroonensis* and *Trichoderma P3* was assess, follow by determination of the inhibition diameters of extracts of *Streptomyces cameroonensis, Trichoderma P3,* and their coculture on *Ralstonia solanacearum* which permitted to develop the most active formulations. The formulations were characterised, and their mode of action and disease severity on detached potato leaves determined using the methods described. GC-MS analysis of the *Trichoderma P3*, the coculture, and *Streptomyces cameroonensis* extracts was done to identify potential bioactive metabolites with antimicrobial activities.

MATERIALS AND METHODS

Materials

Trichoderma sp. *P3* was provided by the Laboratory for Phytobiochemistry and Medicinal plant studies of the University of Yaoundé I, Cameroon. The bacteria *Ralstonia solanacearum* and *Streptomyces cameroonensis;* the oomycete *Pythium myriotylum;* and the fungi *Phytophthora infestans* and *Fusarium oxysporum* were obtained from the microorganism bank of the Laboratory of Phytoprotection and Valorization of Genetic Resources of the Biotechnology Center, University of Yaoundé I, Cameroon.

Methods

Study of the compatibility of *Streptomyces cameroonensis* and *Trichoderma P3* strains

The compatibility of the *Streptomyces cameroonensis* and *Trichoderma* sp. *P3* strains was determined by dual culture *in vitro* on potato dextrose agar (PDA) medium **(Soares *et al.*, 2006)**. The actinobacteria was seeded on the PDA medium 1.5 cm from the edge of the 90 mm diameter Petri dish. These cultures were incubated at 28 ± 2°C for 5 days. After incubation, a 6 mm diameter disk of 8-day-old fungus culture was placed 1.5 cm from the edge of the petri dish, opposite the actinobacteria culture. For each strain, a control treatment was carried out by placing a fungal disk on the PDA medium free of actinobacterial isolates and inoculating the latter in the absence of the fungal. All plates were incubated at 28 ± 2°C for 28 days. After 4 weeks, the viability of the microorganisms was checked by inoculating the union zone on culture medium supplemented with bactericide firstly and fungicide secondly.

Evaluation of the antagonistic effect of strains of *Streptomyces cameroonensis and Trichoderma P3* and their coculture against phytopathogens

The screening of the antagonistic effect of *Streptomyces cameroonensis, Trichoderma P3* and their coculture against the fungi *Pythium myriotylum, Phytophthora infestans,* and *Fusarium oxysporum* was determined by *in vitro* dual culture **(Soares *et al.*, 2006)**. The inhibition diameter of *Ralstonia solanacearum* was determined using the agar cylinder method described by **Ekundayo and Oyeniran (2016)**. Thus, the percentage inhibition of fungal growth by *Streptomyces cameroonensis, Trichoderma* spp. *P3,* and their coculture was determined everyday for 10 days, while the diameter of inhibition of *Ralstonia solanacearum* was measured after 24 hours.

Production of secondary metabolites of *Streptomyces cameroonensis, Trichoderma P3* strains, and their coculture

Preculture and cultivation of these strains were carried out according to the modified (substrate of solid fermentation) protocol of **Selvameenal *et al.* (2009)**. The preculture of *Streptomyces cameroonensis* and the preculture of *Trichoderma P3* on liquid medium made it possible to have fermentations on solid medium (rice) at 28±2 °C for 30 days of the strains and their coculture. The method described by **Selvameenal *et al.* (2009)** was used to extract metabolites using hexane and ethyl acetate as solvents.

The activities of the extracts of *Streptomyces cameroonensis, Trichoderma P3* and their coculture on *Ralstonia solanacearum.*

The inhibition diameter of the extracts was determined according to the method described by **Ekundayo *et al.* (2016)**, using the extracts at 100 mg/mL.

Determination of the minimum inhibitory concentration (MIC) of the crude extracts of *Streptomyces cameroonensis, Trichoderma P3* and their coculture on *Ralstonia solanacearum.*

The minimum inhibitory concentrations of the hexane and ethyl acetate extracts of *Streptomyces cameroonensis, Trichoderma* P3*,* and their coculture were determined according to the protocol described by **Hassan *et al.* (2009)**. Thus, among the hexane and ethyl acetate extracts, the most active was selected for the continuation of the work.

Formulation

The formulation employed consisted of the three-factor mixture matrix (table 1), incorporating the *Trichoderma P3* extract, the emulsifier Tween 80, and the coculture extract to determine the optimal ratio of the emulsifier and the two extracts for optimal stability and activity against *Ralstonia solanacearum* (inhibition diameter). The different mixtures were prepared after dissolving the extracts in distilled water at 10%.

The stability and activity models of the emulsions were established. Experimental data for each response variable were fitted to the:

- linear model ($f(x) = \beta + a\mathrm{Tr} + b\mathrm{Tw} + c\mathrm{Co}$);
- quadratic model ($f(x) = \beta + a\mathrm{Tr} + b\mathrm{Tw} + c\mathrm{Co} + d\mathrm{Tr}^*\mathrm{Tw}^* + e\mathrm{Tr}^*\mathrm{Co} + f\mathrm{Tw}^*\mathrm{Co}$);
- special cubic model ($f(x) = \beta + a\mathrm{Tr} + b\mathrm{Tw} + c\mathrm{Co} + d\mathrm{Tr}^*\mathrm{Tw}^* + e\mathrm{Tr}^*\mathrm{Co} + f\mathrm{Tw}^*\mathrm{Co} + g\mathrm{Tr}^*\mathrm{Tw}^*\mathrm{Co}$);
- cubic model ($f(x) = \beta + a\mathrm{Tr} + b\mathrm{Tw} + c\mathrm{Co} + d\mathrm{Tr}^*\mathrm{Tw}^* + e\mathrm{Tr}^*\mathrm{Co} + f\mathrm{Tw}^*\mathrm{Co} + g\mathrm{Tr}^*\mathrm{Tw}^*\mathrm{Co} + h\mathrm{Tr}^*\mathrm{Tw}\,(\mathrm{Tr}\text{-}\mathrm{Tw}) + i\mathrm{Tr}^*\mathrm{Co}\,(\mathrm{Tr}\text{-}\mathrm{Co})$).

where, f(x) is the response (stability or activity); β is the constant; Tr, Tw and Co are independent variables and a, b, c, d, e, f, g, h, and i are the coefficients.

The most significant stability and activity models were retained, and based on these data, a formulation (100 mL) was developed based on TrP3 (10g Tr+ 70mL Tw + 20 mL dH_2O) and another based on the coculture (10g Coculture+70 mL Tw + 20mL dH_2O).

Table 1 Code of the 3-factor Simplex lattice design and the corresponding amount (mL)of the extracts in the mixture

Mixtures	*Trichoderma* P3(TrP3)	Tween80	Coculture TrP3+*S.cameroonensis*
1	1	0	0
2	0	1	0
3	0	0	1
4	0.5	0.5	0
5	0.5	0	0.5
6	0	0.5	0.5
7	0.667	0.167	0.167
8	0.167	0.667	0.167
9	0.167	0.167	0.667
10	0.33	0.33	0.33

Characterization of the best formulations
Droplet size and pH

The diameter of the droplets of the two stable formulations with high biological activity, TrP3 (10%) and the coculture (10%) respectively, as well as their pH, were determined as described by **Foka *et al.* (2023)**.

Thermodynamic stability

The thermodynamic stability was evaluated first by centrifugation and then by temperature variation. The formulations were centrifuged at 1000 x g for 30 minutes at 25°C, and any phase separation was reported **(Dhivya *et al.*, 2019)**. The effect of temperature on the stability of the formulations was studied at 45°C and -6°C as described by **Dhivya *et al.* (2019)**. The formulations were observed to identify any phase separations.

Biological activity of the two formulations
Determination of the inhibitory effect of formulations based on *Trichoderma P3* and the coculture on the growth of *R. solanacearum.*

The inhibition diameters and the MICs of the formulations based on *Trichoderma P3* and the coculture *Trichoderma P3/Streptomyces cameroonensis* against *R. solanacearum* were determined as previously described **(Ekundayo *et al.*, 2016; Hassan *et al.*, 2009)**.

Mode of action of the formulations

The formulations were employed to investigate their possible modes of action (bacteriolysis, inhibition of protein synthesis, and inhibition of ATPase-H+ pumps) against *Ralstonia solanacearum*. The lytic action, inhibition of protein synthesis, and inhibition of ATPase-H+ pumps were done according to the protocols established by **Kalia *et al.* (2009)**, **Limsuwan and Voravuthikunchai (2013)**, and **Manavathu *et al.* (2001)**, respectively.

Effect of the formulations on the severity of the potato disease caused by *Ralstonia solanacearum.*

The effect of the formulations at 20%, 5% and 1.25% was evaluated against the severity of the potato disease caused by *Ralstonia solanacearum* using the detached leaf protocol described by **Zhang *et al.* (2015)**. The experiment was performed twice, and the disease severity index was determined. Disease expression was assessed six days after inoculation, using the rating scale developed by **Nyassé *et al.* (1995)**.

Gas chromatography analysis of the sample extract of *Trichoderma P3, Streptomyces cameroonensis* and their coculture

The ethyl acetate extract of *Trichoderma P3, Streptomyces cameroonensis* and their coculture was analysed following **Shasthree *et al.* (2022)** modified methodology. The GC-MS analysis used an Agilent Technology GC system 7890A system coupled with an Agilent Technology MS 5975C VLMSD. The capillary column Agilent 190915-433 325ºC: 30 m x 250 µm x 0.25 µm was used for GC separation. The carrier gas was helium (He) at a flow rate of 0.8 mL.min-1. The column temperature was maintained at 80 ºC for 1 min, followed by changes of 8 ºC/min up to 200 °C and then 1 min; followed by changes of 8 ºC/min up to 280 °C/min and then 6 min at 280 ºC. The total cycle time was 33 min., and the pressure was 7.378 psi. The inlet and detector temperatures were 150 ºC and 230 ºC respectively. The total peak area was used to calculate the percentage of each bioactive compound in the bioformulation. The GC-MS Analyzer was equipped with GC-MS NIST-II library. Each metabolite's relative amount was calculated by comparing its average peak area to the overall area. Identification of the isolated volatile metabolites was done using retention indices and mass spectrometry with the NIST library database.

Statistical Analyses

For the compatibility of the *Streptomyces cameroonensis* and *Trichoderma* P3 strains, the inhibition diameters of the of extracts of *Streptomyces cameroonensis, Trichoderma P3,* and their coculture on *Ralstonia solanacearum*, the effect of the formulations based on *Trichoderma P3* extract (*TrP3)* and Coculture extract *(Coculture TrP3 + S. cameroonensis)* on the MIC of *R. solanacearum* and mode of action, and the variation of disease severity caused by *R. solanacearum* were determined and results expressed as means ± standard deviation. The Analysis of Variance (ANOVA) and significant differences were established using the Tukey's test with *P* value less than 0.05. STATISTICA 10 software was used to develop the validity of the stability and activity models.

RESULTS AND DISCUSSION

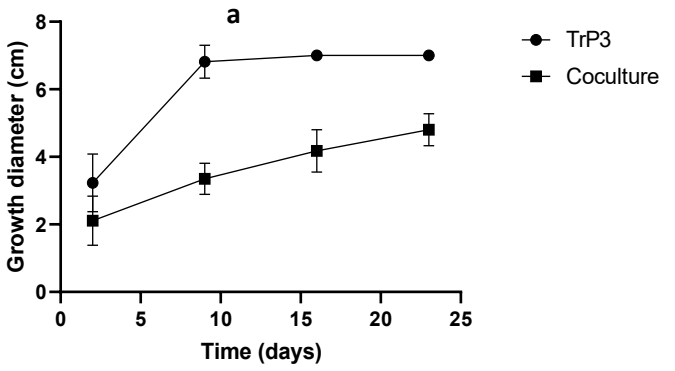
Results

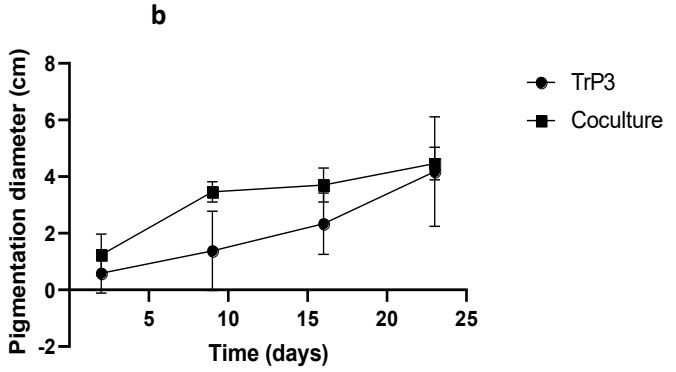
Compatibility of the *Streptomyces cameroonensis* and *Trichoderma P3* strains and their effect on phytopathogens

The confrontation between *Streptomyces cameroonensis* and *Trichoderma P3* revealed that *Streptomyces cameroonensis* has a significant inhibitory effect on the growth of *Trichoderma P3* but does not have a fungicidal effect on the latter (figure 1a). However, inhibition of the growth of *Trichoderma P3* by *Streptomyces cameroonensis* leads to an induction of early production of green and yellowish pigments by the latter (figure 1b).
The confrontation of fungal phytopathogens in the presence of *Trichoderma P3* and the *Trichoderma P3+ Streptomyces cameroonensis* coculture on PDA revealed that the latter have a significant inhibitory effect on the development of fungal pathogens and that *F. oxysporum* is the most sensitive (figure 2). Furthermore, the inhibition diameter of *Ralstonia solanacearum* using agar cylinders showed no significant difference between *Trichoderma P3* and the coculture on *Ralstonia solanacearum* (figure 3).

Determination of the inhibition diameters of extracts of *Streptomyces cameroonensis, Trichoderma P3,* and their coculture on *Ralstonia solanacearum*

The extracts of *Streptomyces cameroonensis, Trichoderma P3,* and their coculture were obtained with yields of 0.782%, 0.6993%, and 0.7847%, respectively, in hexane and 2.0327%, 4.83%, and 4.14%, respectively, in ethyl acetate.
The activity of the extracts of *Streptomyces cameroonensis, Trichoderma P3,* and their coculture on *Ralstonia solanacearum* revealed that extracts with ethyl acetate were more effective than those with hexane (Figure 4a). In addition, the MICs shows that there is no significant difference between *Trichoderma P3* and the coculture (Figure 4b).





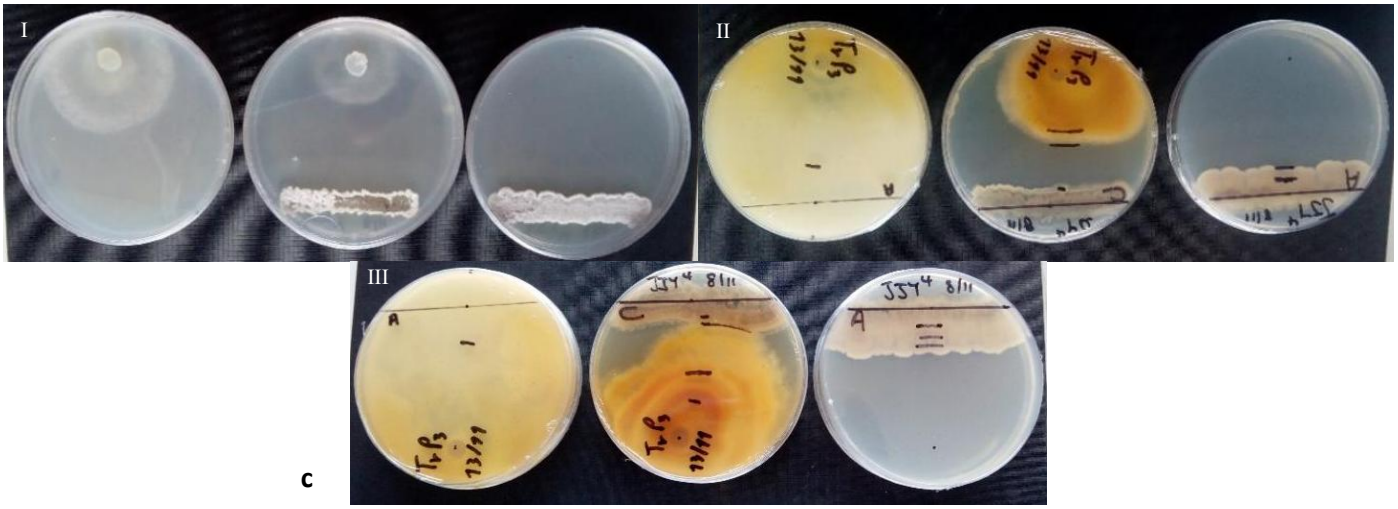


Figure 1 Confrontation of *Streptomyces cameroonensis* and *Trichoderma* P3. **a**: growth diameter of the mycelium. **b**: pigmentation diameter of the mycelium. **c**: images of *Trichoderma* P3, coculture and *Streptomyces cameroonensis* at 2 days (I), 14 days (II), and 28 days (III). TrP3 – *Trichoderma P3, Coculture – coculture Trichoderma P3+ Streptomyces cameroonensis*

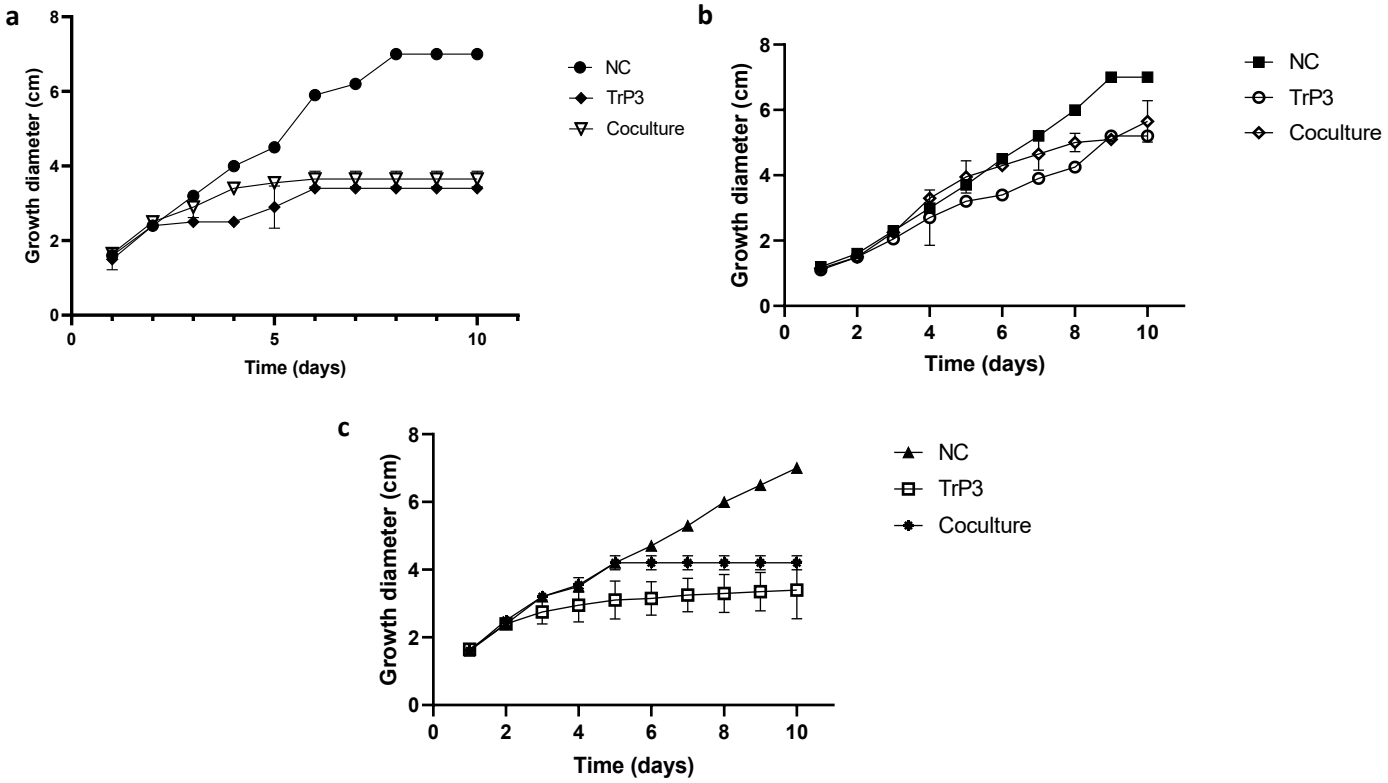


Figure 2 Growth of fungal phytopathogens in the presence of *Trichoderma P3* and the *Trichoderma P3+ Streptomyces cameroonensis* coculture. **a**: *Fusarium oxysporum.* **b**: *Pythium myriotylum.* **c**: *Phytophthora infestans*. NC – Negative control, TrP3 – *Trichoderma P3, Coculture – coculture Trichoderma P3+ Streptomyces cameroonensis*

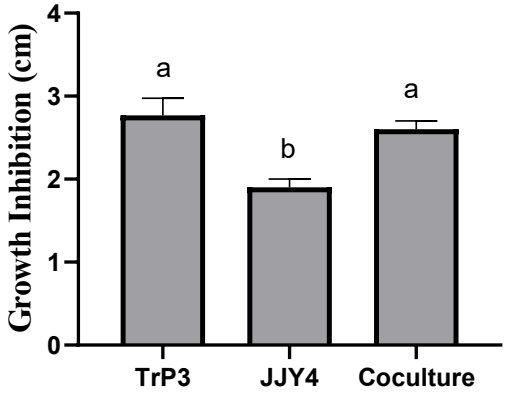


Figure 3 Growth inhibition diameter of *Ralstonia solanacearum. TrP3: Trichoderma P3: JJY4: Streptomyces cameroonensis; Coculture: Coculture TrP3 + JJY4.* The means with different letter are significantly different at $P<0.05$

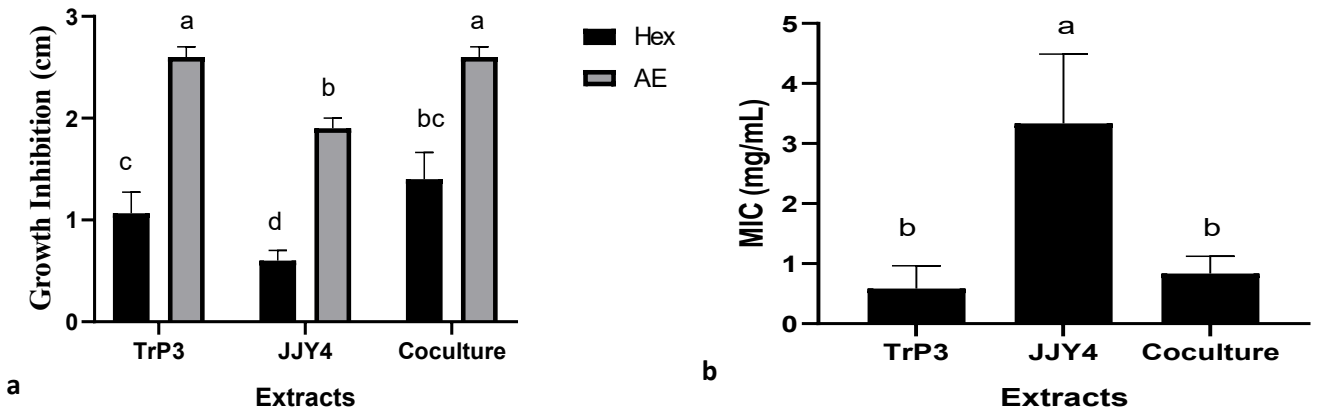


Figure 4 Activities of the extracts against *Ralstonia solanacearum.* **a**: diameter of the inhibition halos. **b**: MIC of ethyl acetate extracts of cultures of *Streptomyces cameroonensis, Trichoderma P3* and their coculture on *Ralstonia solanacearum.* TrP3 – *Trichoderma P3,* JJY4 – *Streptomyces cameroonensis;* Coculture – *Coculture TrP3 + JJY4.* The means with different letters are significantly different at *P<0.05*

Formulation

The matrix of the three-factor mixtures (*Trichoderma P3*, Tween80, and the coculture *Streptomyces cameroonensis* JJY4^T-*Trichoderma P3*) from the extracts revealed that the cubic prediction is the most appropriate for the evolution of stability and inhibitory activity against *Ralstonia solanacearum* (table 2, figure 5, and figure 6). Table 2 shows the coefficients of the most significant models (P=0.00001 and P= 0.000003 for stability and activity cubic models, respectively) of stability and activity.

Furthermore, fitted surface (figure 5a) and trace plots (figure 5b) of cubic stability model response of the 3 factors (TrP3, Tween80 and Coculture) of the simplex-lattice design show that Tween80 has a positive effect on the stability. Meanwhile, TrP3 and coculture have the best positive effects on the activity against *Ralstonia solanacearum* observing the fitted surface (figure 6a) and trace plot (figure 6b).

Table 2 Parameters of the stability and activity models tested

Parameters of the stability models										
Model	SS effect	Df effect	MS effect	SS error	Df error	MS error	F	p	R-Sqr	R-Sqr adjusted
Linear	35208.34	2	17604.17	18229.16	27	675.1541	26.07430	0.000000	0.658870	0.633601
Quadratic	13522.50	2	6761.25	4706.66	25	188.2664	35.91320	0.000000	0.911922	0.897830
Special Cubic	253.76	1	253.76	4452.90	24	185.5376	1.36769	0.253692	0.916671	0.899311
Cubic	2894.51	2	1447.25	1558.40	22	70.8362	20.43097	0.000010	0.970837	0.961558
Parameters of the activity models										
Model	SS effect	Df effect	MS effect	SS error	Df error	MS error	F	p	R-Sqr	R-Sqr adjusted
Linear	13.78482	2	6.892409	3.133848	27	0.116068	59.38228	0.000000	0.814770	0.801049
Quadratic	0.61824	2	0.309122	2.515604	25	0.100624	3.07205	0.064129	0.851312	0.827522
Special Cubic	0.21193	1	0.211926	2.303678	24	0.095987	2.20787	0.150327	0.863838	0.835471
Cubic	1.56954	2	0.784771	0.734136	22	0.033370	23.51739	0.000003	0.956608	0.942801

Parameters of the cubic stability model						
Factor	Coef.	Std.Err.	t (21)	p	-95. %	+95. %
(A)TrP3	1.593	4.9533	0.32163	0.750910	-8.708	11.8940
(B)Tween80	101.593	4.9533	20.51037	0.000000	91.292	111.8940
(C)Coculture	1.593	4.9533	0.32163	0.750910	-8.708	11.8940
AB	206.373	24.2858	8.49766	0.000000	155.867	256.8777
AC	6.373	24.2858	0.26240	0.795572	-44.133	56.8777
BC	206.373	24.2858	8.49766	0.000000	155.867	256.8777
ABC	211.765	159.2952	1.32939	0.197983	-119.508	543.0373
AB(A-B)	500.000	80.6789	6.19740	0.000004	332.219	667.7806
AC(A-C)	-250.000	80.6789	-3.09870	0.005439	-417.781	-82.2188

Parameters of the cubic activity model						
Factor	Coef.	Std.Err.	t (21)	p	-95, %	+95, %
(A)TrP3	3.1845	0.105207	30.26864	0.000000	2.9663	3.40267
(B)Tween80	0.5182	0.095771	5.41077	0.000020	0.3196	0.71681
(C)Coculture	2.7515	0.095771	28.73021	0.000000	2.5529	2.95015
AB	-1.1295	0.508857	-2.21970	0.037057	-2.1848	-0.07420
AC	-0.5295	0.508857	-1.04058	0.309370	-1.5848	0.52580
BC	-8.2820	3.093722	-2.67702	0.013770	-14.6980	-1.86598
ABC	-11.7341	1.711463	-6.85617	0.000001	-15.2834	-8.18473
AB(A-B)	5.6659	1.711463	3.31056	0.003181	2.1165	9.21526
AC(A-C)	3.1845	0.105207	30.26864	0.000000	2.9663	3.40267

The equation of the stability and activity model are as follows:

$Stability_{(x,y,z)}$=+2,1311393032995*x+101,59835327871*y+2,1311393032996*z+205,32786024589*x*y+205,32786024589*y*z+228,6885518844*x*y*z+502,13079836112*x*y*(x-y)-251,06539918056*x*z*(x-z)

$Activity_{(x,y,z)}$=+3,1844809493987*x+,51819680590143*y+2,7515301392348*z-1,1295081016393*x*y-,52950810163925*x*z-8,28196682163*x*y*z-11,734089547885*x*y*(x-y)+5,6658990521372*x*z*(x-z)+0,

Where x= *Trichoderma* P3; y=Tween80; z= coculture *Trichoderma P3* + *Streptomyces cameroonensis JJY4*.

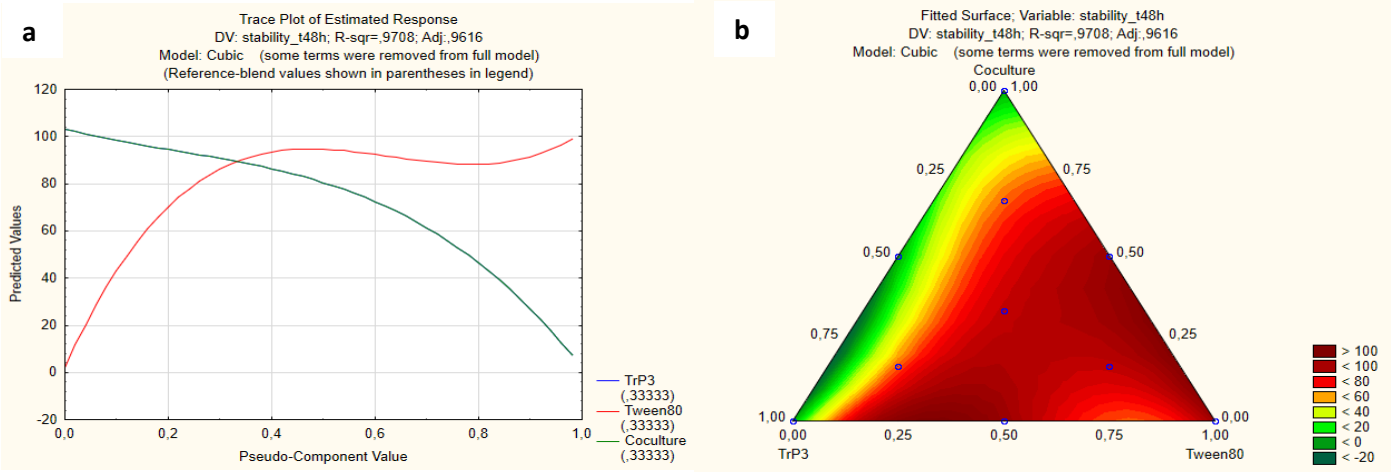


Figure 5 Fitted surface (**a**) and Trace plot (**b**) of cubic stability model response of the 3 factors of the simplex-lattice design. TrP3, Tween80 and Coculture are the independent factors

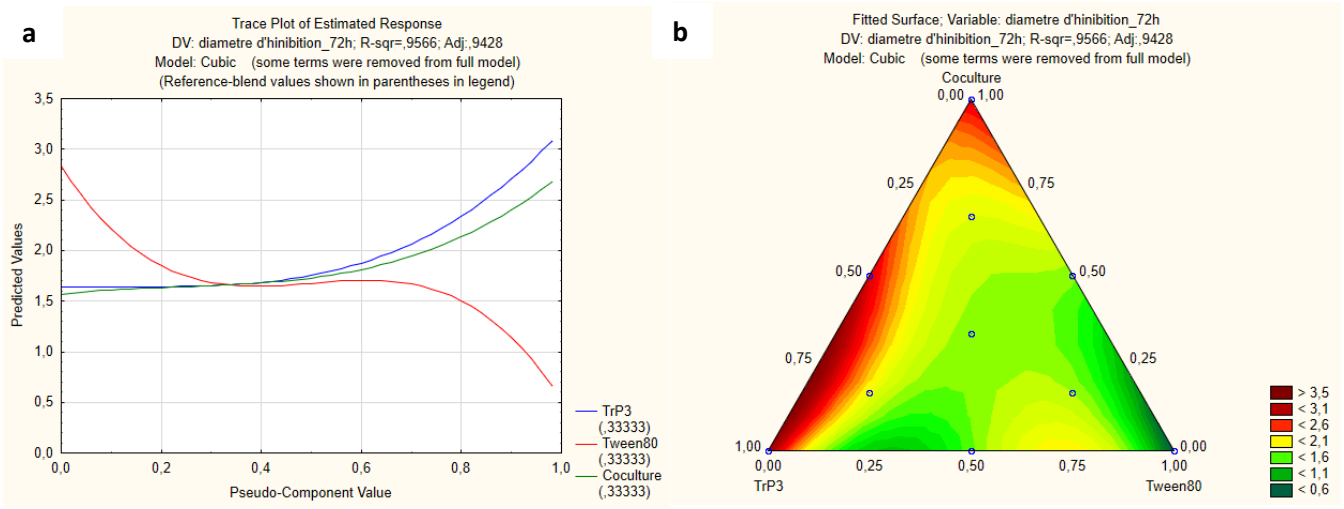


Figure 6 Fitted surface (**a**) and Trace plot (**b**) of cubic model activity (halo inhibition of *Ralstonia solanacearum*) response of the 3 factors of the simplex-lattice design. TrP3, Tween80 and Coculture are the independent factors

Characterization of the formulations

The two formulations (TrP3 and the one with Coculture) were stable at 25°C. The formulation with TrP3 and the one with coculture were acidic despite surfactant concentration, that is pH, 4.1 and 4.3, respectively (table 3). The microscopic image of these formulations revealed the spherical shape of the droplet, and it also confirmed the micro size of the droplet, which was less than 5.36 µm (table 3). Table 5 also shows that centrifugation of the formulation at 1000 ×g at 25°C for 30 minutes does not influence the stability. The thermal stability of the formulations was also observed at 45°C (table 3). The formulations *Trichoderma P3* and its coculture with *Streptomyces cameroonensis* gave the halo of 1.86 and 1.78 against *Ralstonia solanacearum,* respectively.

The evaluation of the MIC against *Ralstonia solanacearum* revealed that the formulations *Trichoderma P3* and its coculture with *Streptomyces cameroonensis* have the same value (4.16 mg/mL) which is significantly higher than that of the positive control streptomycin, which is 0.16 mg/mL (figure 7).

Table 3 Thermodynamic stability and activity against *Ralstonia solanacearum* of the formulation with *Trichoderma P3* and its coculture with *Streptomyces cameroonensis* JJY4^T

Formulations	Shape	droplet size [µm]	pH	Centrifugation at 1000 ×g	thermal stability at 45°C	thermal stability at -6 °C	growth Inhibition of *R. solanacearum* (cm)
TrP3	Spherical	≤ 5.36 ±1.00	4.1 ± 0.15	Stable	Stable	Stable	1.86 ± 0.12
Coculture	Spherical	≤ 5.36 ±1.00	4.3 ± 0.20	Stable	Stable	Stable	1.78 ± 0.12

TrP3 – Trichoderma P3, Coculture – Coculture TrP3 + S. cameroonensis.

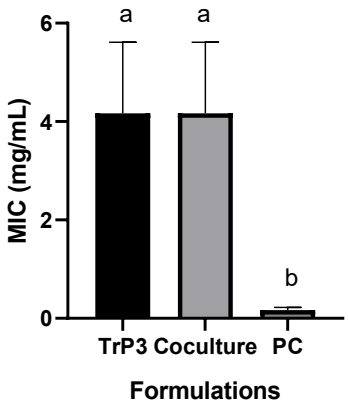


Figure 7 Effect of the formulations based on *Trichoderma P3* extract (*TrP3)* and Coculture extract *(Coculture TrP3 + S. cameroonensis)* on the MIC of *R. solanacearum.* The means with different letters are significantly different at *P<0.05*

Mode of action of the formulations on *R. solanacearum*

The study of the mode of action of the *Trichoderma* P3 and the coculture *Trichoderma* P3+ *Streptomyces cameroonensis* bioformulations revealed that they had lytic effects, inhibited protein synthesis and the ATPase-H+ pump of the phytopathogen *R. solanacearum* (figure 8). The formulation based on the Coculture (1 MIC and 0.5 MIC) and TrP3 (0.5 MIC) showed a lytic effect on the wall of the bacterium in contrast to TrP3 (1MIC) after 24 hours (figure 8A). Furthermore, the Coculture TrP3+JJY4 (1 MIC and 0.5 MIC) and that of TrP3 (1 MIC) led to an increase in protein synthesis by *R. solanacearum,* unlike that of TrP3 (0.5 MIC) which did not affect this parameter (figure 8B). However, the two formulations at 1MIC and 0.5 MIC significantly affected the activity of the ATPase-H+ pump of the plant pathogen *R. solanacearum* (figure 8C).

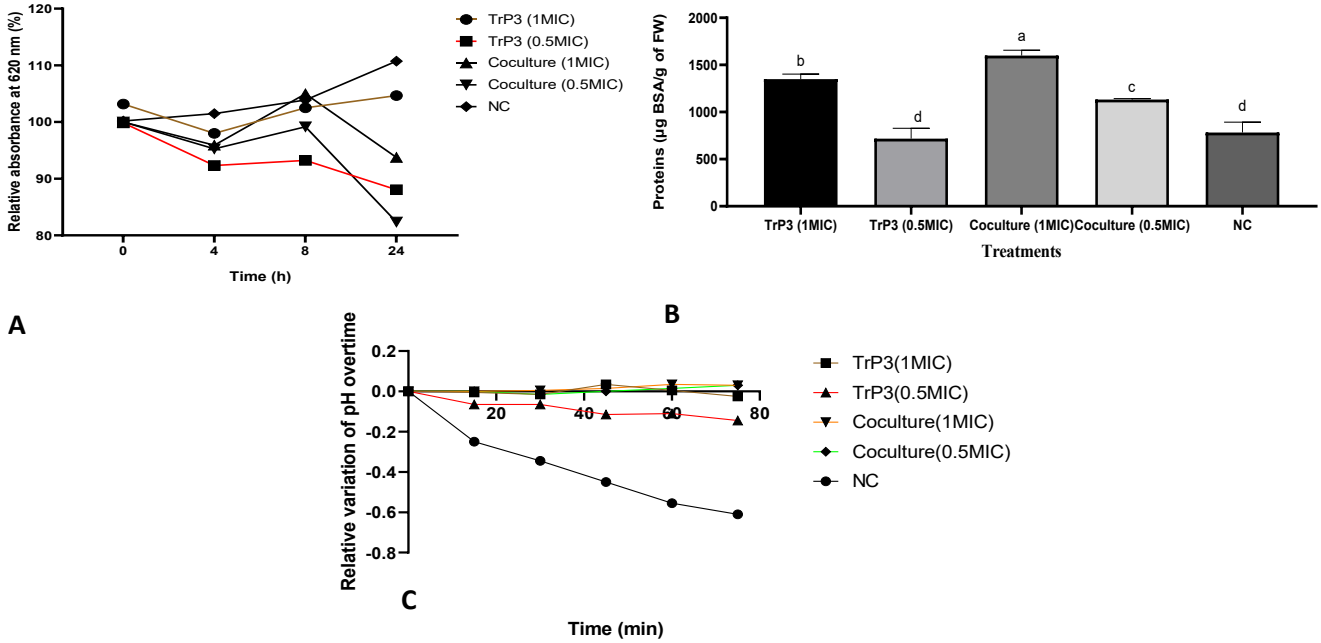


Figure 8 Mode of action of the formulations based on *Trichoderma P3* extract (*TrP3)* and Coculture extract *(Coculture TrP3 + S. cameroonensis),* on *R. solanacearum*. A: cell wall integrity; B: Protein synthesis by pathogen; C: ATPase-H^+ pumps activities. NC – negative control, TrP3 – *Trichoderma* P3, *Coculture – TrP3 + S. cameroonensis*

Disease severity index

Pretreatment of detached potato leaves with the TrP3 and Coculture TrP3+JJY4^T formulations led to a variation in the severity of the infection (figure 9). The treatment of leaves with Tr20% led to a reduction in the severity of the infection from 2 to 1.16, while Coculture 20% reduced it to 1.33.

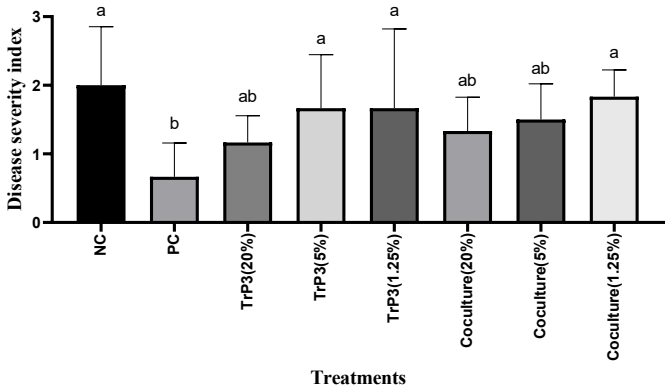
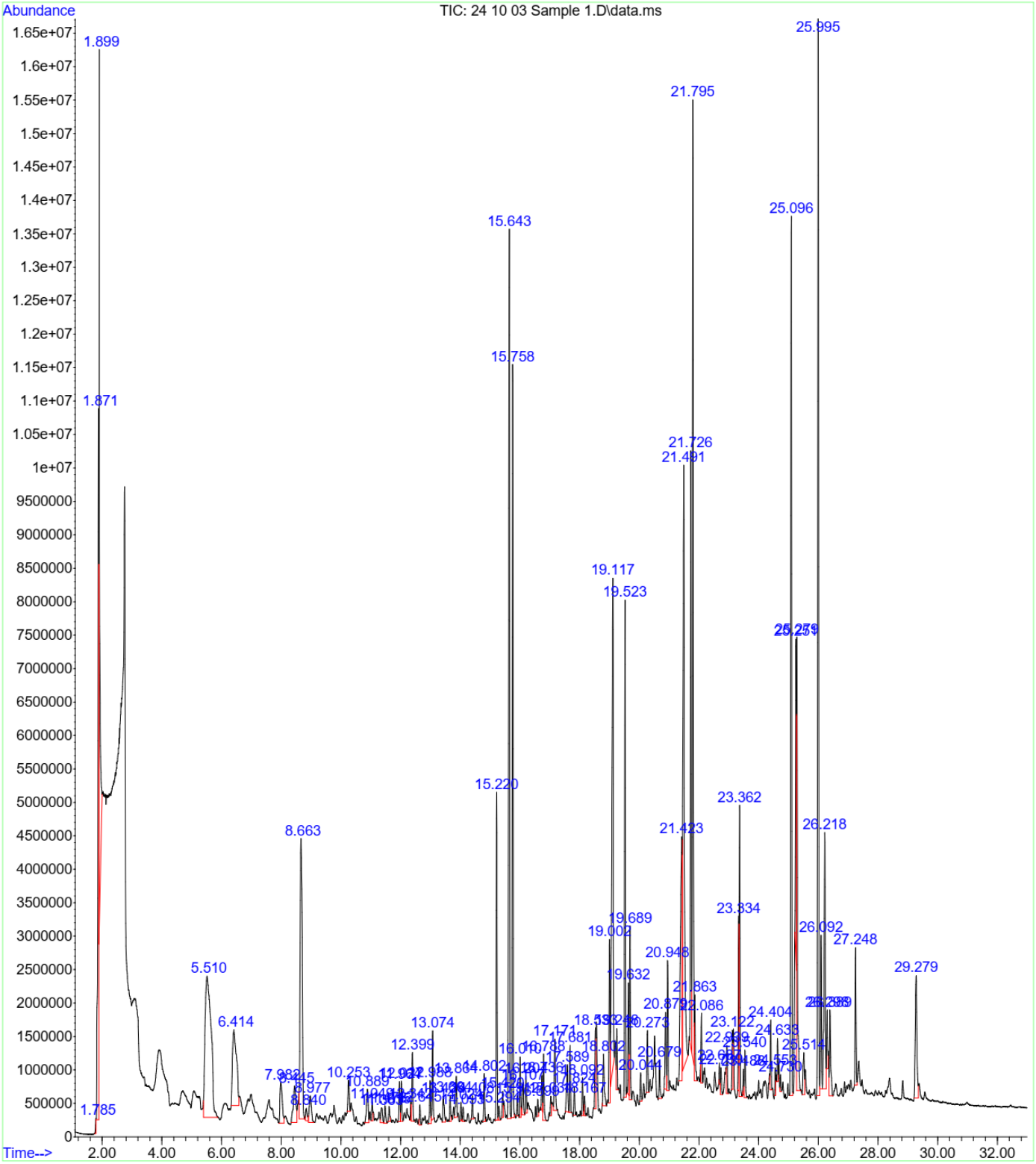


Figure 9 Variation of disease severity caused by *R. solanacearum* according to different treatments. NC – negative control, PC – positive control, TrP3 – *Trichoderma P3*, Coculture – coculture of *Trichoderma P3+ S. cameroonensis*. The means with different letters are significantly different at *P<0.05*

Gas chromatography analysis of the sample extract of *Trichoderma P3, Streptomyces cameroonensis* and their coculture

The GC-MS analysis of the *Trichoderma P3, Streptomyces cameroonensis* and their coculture extracts shows the presence of 91, 80 and 61 volatile compounds respectively in the (figure 10). The major compounds present in each extract are summarized in table 4 below. Between those compounds, we have some which are present only in the *Trichoderma P3* extract (5,6-Decanediol; 9-Nonadecene); those present in the coculture extract only (4-Imidazolidinone, 5-(phenylmethyl)-2-thioxo-; 9-Octadecenoic acid (Z)-, methyl ester); those present in the *Streptomyces cameroonensis* extract only (1-Docosene; 1-Octadecene; 9-Octadecene, (E)-) those present in *Trichoderma P3* and the coculture extract (3-Octadecanone; 9,12-Octadecadienoic acid (Z,Z)-; 9,12-Octadecadienoic acid, methyl ester); those present in *Streptomyces cameroonensis* and the coculture only (Hexadecanoic acid, methyl ester); and those present in the *Trichoderma P3, Streptomyces cameroonensis* and their coculture (1-Dodecene; Oleic Acid) (table 4).


Abundance
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Time-->
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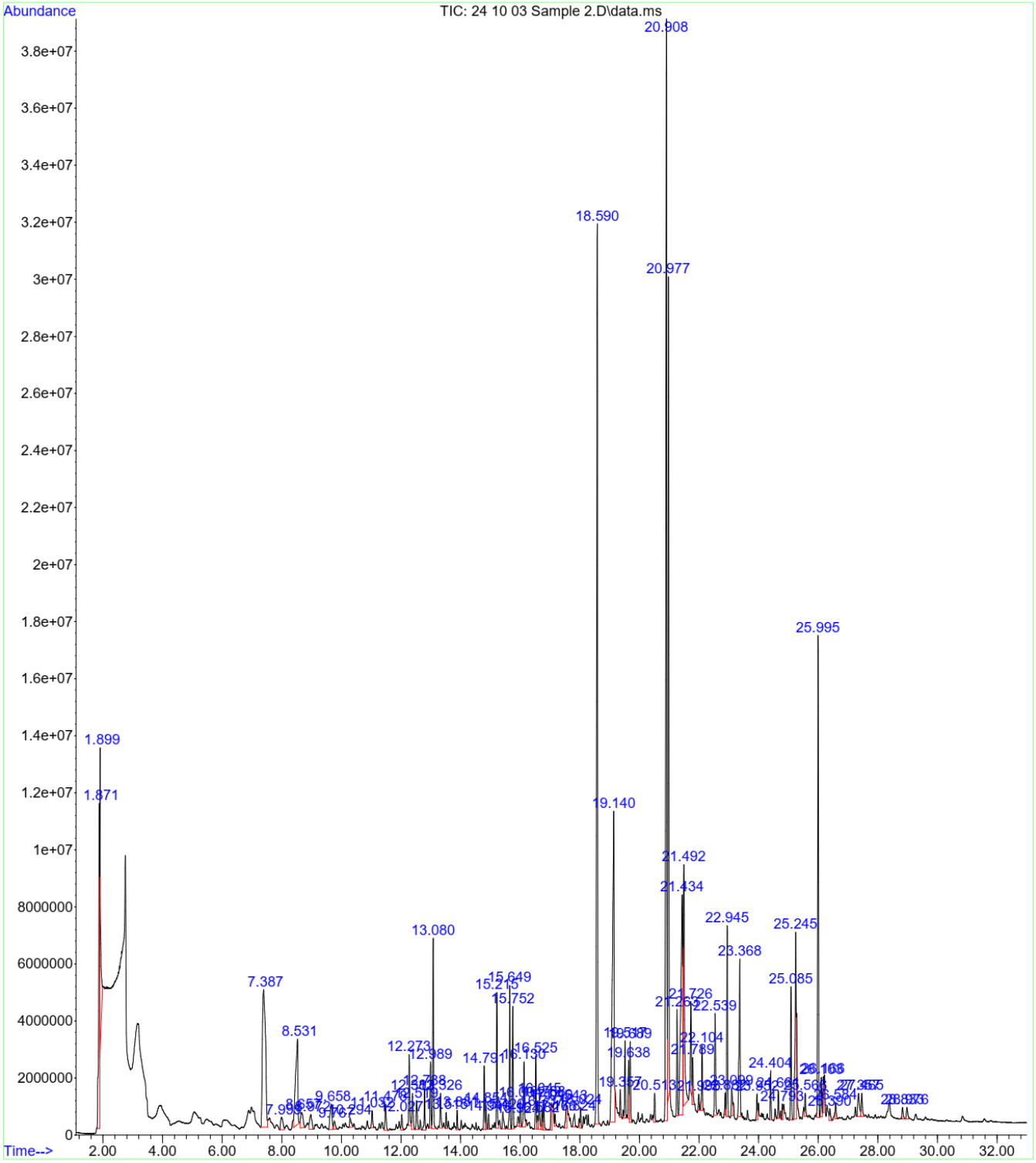
1.871
1.785
25.995
21.795
25.096
15.643
15.758
21.726
21.491
19.117
19.523
15.220
23.362
8.663
21.423
26.218
23.334
19.689
19.002
26.092
27.248
20.948
5.510
29.279
19.632
6.414
13.074
12.399

a


Abundance
TIC: 24 10 03 Sample 2.D\data.ms
Time-->
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20.977
25.995
1.899
1.871
19.140
21.492
21.434
22.945
25.245
23.368
13.080
25.085
7.387
15.649
15.215
15.752
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22.539
22.104
12.273
12.989
14.791
16.525
16.130
19.638
24.404

b

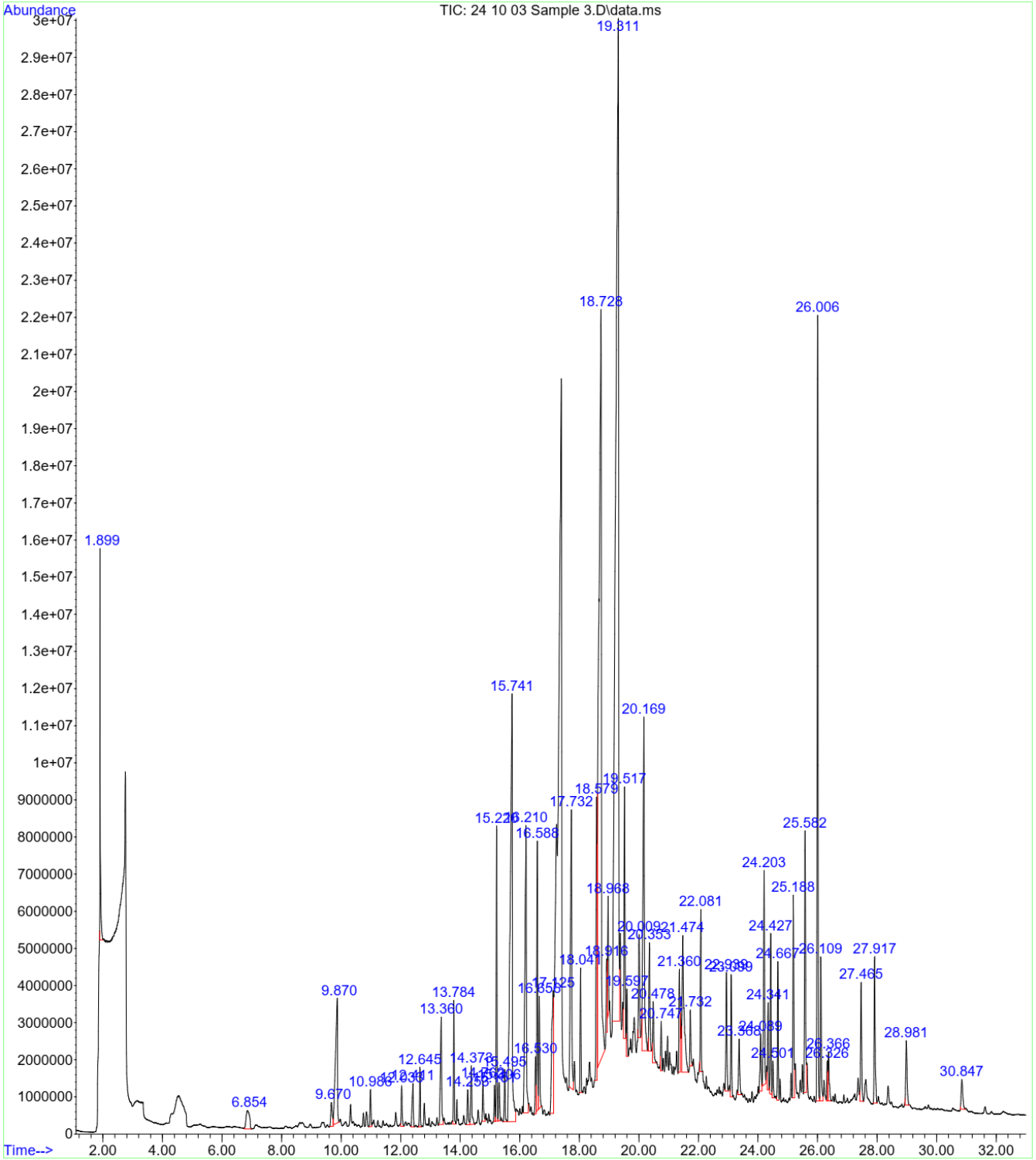

Figure 10 GC-MS chromatogram of the *Trichoderma P3* extract (A); Co-culture of *Trichoderma P3* and *Streptomyces cameroonensis* extract (B); *Streptomyces cameroonensis* extract (C). Each number on the peaks represents the retention time of the compounds.

Table 4 Comparative analysis of the major compounds in the *Trichoderma P3* extract, Coculture of *Trichoderma P3* and *Streptomyces cameroonensis* and *Streptomyces cameroonensis* extract. The area in bold was shown as the main compounds present in the extract and has an abundance up to 5000000

R T (min ± 0.1)	Probable Compound names	MF	MW g/mol	*Trichoderma P3* extract (Area %)	Coculture extract (Area %)	*Streptomyces cameroonensis* extract (Area %)
13.080	Benzeneacetic acid, ethyl ester	$C_{10}H_{12}O_2$	164.2	0,28	**0.65**	-
15.220	1-Dodecene	$C_{12}H_{24}$	168.32	1.67	1.24	**1.67**
15.643	5,6-Decanediol	$C_{10}H_{22}O_2$	174.28	**5.89**	-	-
15.649	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.4	-	**1.33**	-
15.741	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	0.24	0.77	**6.94**
15.758	Benzene, (3-nitropropyl)-	$C_9H_{11}NO_2$	165.19	**4.37**	-	-
17.732	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	0.12	0.13	**1.69**
18.590	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	-	**10.73**	2.12
19.117	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	**4.6**	6.22	13.56

19.517	1-Octadecene	$C_{18}H_{36}$	252.5	-	-	**1.74**
19.523	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5	**2,78**	0.73	-
20.169	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.5	0.17-	-	**3.52**
20.353	9-Octadecene, (E)-	$C_{18}H_{36}$	252.5	-	-	**0.71**
20.908	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	0.52	**12.96**	-
20.977	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.5	-	**8.12**	-
21.434	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	0,13	**2,46**	-
21.491	Oleic Acid	$C_{18}H_{34}O_2$	282.5	**5.63**	3.12	1.91
21.726	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.5	**3.35**	0.99	-
21.795	Ethyl Oleate	$C_{20}H_{38}O_2$	310.5	**5.60**	0.45	-
22.081	1-Docosene	$C_{22}H_{44}$	308.6	-	-	**0.94**
22.945	Butyl citrate	$C_{18}H_{32}O_7$	360.4	-	**0.61**	-
23.368	3-Octadecanone	$C_{18}H_{36}O$	268.5	1,55	**1.98**	-
24.427	Diisooctyl adipate	$C_{22}H_{42}O_4$	370.6	-	-	**0.98**
25.085	4-Imidazolidinone, 5-(phenylmethyl)-2-thioxo-	$C_{10}H_{10}N_2OS$	206.27	-	**1,29**	-
25.096	Butane, 1-(benzyloxy)-2-[(benzyloxy)methyl]-	$C_{19}H_{24}O_2$	284.4	**5.46**	-	-
25.188	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5	-	-	**0.88**
25.279	9-Nonadecene	$C_{19}H_{38}$	266.5	**2.42**	-	-
25.582	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5	-	-	**0.88**
26.006	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.6	6.39	4,73	**5.57**
7.387	Benzeneacetic acid, methyl ester	$C_9H_{10}O_2$	150.17	-	**4.96**	-

Among the eighty compounds present in the coculture of *Trichoderma P3* and *Streptomyces cameroonensis,* forty compounds were absent in the two single cultures of these microorganisms (table 5). Between these compounds, we have antibacterial, antimicrobial, antioxidant, antidiabetic, neuroprotective, and anticancer compounds.

Table 5 Induced compounds in the Coculture of *Trichoderma P3* and *Streptomyces cameroonensis*

R T (min)	Probable Compound names	Area (%)	MF	MW g/mol	Activities
7.387 min	Benzeneacetic acid, methyl ester	4.96	$C_9H_{10}O_2$	150.17	Antioxidant
7.993 min	Thiophene, 2,5-dihydro-	0.33	C_4H_6S	86.16	Antimicrobial Activity; Anticancer Properties;
8.972 min	Metformin	0.38	$C_4H_{11}N_5$	129.16	Improve glycemic control
9.767 min	Pentanoic acid, 2-methylpropyl ester	0.14	$C_9H_{18}O_2$	158.24	Antimicrobial Activities; Anti-inflammatory, neuroprotective
10.294 min	Benzaldehyde, 3-hydroxy-	0.16	$C_7H_6O_2$	122.12	Enzyme Inhibitors, Antimicrobial
11.032 min	1-Decene	0.21	$C_{10}H_{20}$	140.27	Antimicrobial (membrane disruption),
11.478 min	Benzeneacetic acid, 2-methylpropyl ester	0.36	$C_{12}H_{16}O_2$	192.25	Antimicrobial, Food Additive: Flavoring Agents
12.273 min	Benzeneacetic acid, 4-hydroxy-, methyl ester	0.77	$C_9H_{10}O_3$	166.17	Antioxydant, Flavoring Agents; antimicrobial
12.382 min	Cycloheptasiloxane, tetradecamethyl-	0.45	$C_{14}H_{42}O_7Si_7$	519.07	Conditioning agents,
12.788 min	Dodecanoic acid, methyl ester	0.43	$C_{13}H_{26}O_2$	214.34	Antimicrobial and antiviral, antioxydant,
13.515 min	Diethyl methylsuccinate	0.16	$C_9H_{16}O_4$	188.22	Drugs delivery
13.881 min	Mono-butyl itaconate	0.18	$C_9H_{14}O_4$	186.2	Anti-inflammatory, Antimicrobial, Tumor Metabolism Modulation
14.791 min	Phenylacetic acid, 3,5-difluorophenyl ester	0.63	$C_{14}H_{10}F_2O_2$	248.22	herbicidal or fungicidal
14.854 min	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	0.22	$C_{16}H_{30}O_4Si_3$	370.66	Antimicrobial Activities; Anti-inflammatory
14.940 min	2-Phenylacetic acid, 2,5-dichlorophenyl ester	0.13	$C_{14}H_{10}Cl_2O_2$	281.1	Antimicrobial Activities
15.420 min	Spiro[4.5]decane, 6-methylene-	0.25	$C_{11}H_{18}$	150.26	Anticancer Properties; Antimicrobial Activity
15.752 min	3-Benzyl-4-bromo-1,2,3-triazole 1-oxide	1.15	$C_9H_8BrN_3O$	254.08	Antimicrobial Activities; Anticancer Properties; Antimicrobial Activity
15.924 min	Phenylacetic acid, pent-2-en-4-ynyl ester	0.14	$C_{13}H_{12}O_2$	200.23	Antimicrobial Activities; Anti-inflammatory
16.004 min	**N'-[(3-Methyl-1H-pyrazol-5-yl)carbonyl]benzenesulfonohydrazide**	0.25	$C_{11}H_{12}N_4O_3S$	280.31	Anticancer Activity; Anti-inflammatory; Antimicrobial
16.525 min	Pentadecanoic acid, methyl ester	0.71	$C_{16}H_{32}O_2$	256.42	Antimicrobial Emollient; Stabilizing Agent
17.543 min	Cyclohexanol, 3,5-dimethoxy-, stereoisomer	0.35	$C_8H_{16}O_3$	160.21	Antioxidant, Antimicrobial, Neuroprotective
19.357 min	2-Hexenedioic acid, bis(trimethylsilyl) ester, (E)-	0.26	$C_{12}H_{24}O_4Si_2$	288.49	Anti-inflammatory
19.638 min	Phthalic acid, nonyl 3-phenylpropyl ester	0.6	$C_{26}H_{34}O_4$	410.5	Endocrine Disruption
19.689 min	Benzene, (3-methyl-1-methylenebutyl)-	0.87	$C_{12}H_{16}$	160.25	Antimicrobial Properties; Endocrine Disruption
20.977 min	9-Octadecenoic acid (Z)-, methyl ester	8.12	$C_{19}H_{36}O_2$	296.5	Antioxidant, Anti-inflammatory
21.263 min	Methyl stearate	0.95	$C_{19}H_{38}O_2$	298.5	Antimicrobial, Anti-inflammatory
21.995 min	Hexadecanoic acid, 1,1-dimethylethyl ester	0.15	$C_{20}H_{40}O_2$	312.5	
22.104 min	Butyl citrate	0.61	$C_{18}H_{32}O_7$	360.4	Plasticizer and additive in food and cosmetic
22.539 min	1-Hydroxy-4-methylanthraquinone	1.19	$C_{15}H_{10}O_3$	238.24	Antimicrobial, Anti-inflammatory, Anticancer
22.882 min	1-Hydroxycyclododecanecarbonitrile	0.21	$C_{13}H_{23}NO$	209.33	Antimicrobial, Anti-inflammatory
22.945 min	Butyl citrate	1.77	$C_{18}H_{32}O_7$	360.4	Biocompatibility, Antimicrobial
24.404 min	Glutaric acid, 6-ethyloct-3-yl isobutyl ester	0.45	$C_{19}H_{36}O_4$	328.5	Antimicrobial, Antifungal, or Insecticidal
25.085 min	4-Imidazolidinone, 5-(phenylmethyl)-2-thioxo-	1.29	$C_{10}H_{10}N_2OS$	206.27	Antimicrobial, Anti-inflammatory, Antioxidant,
26.103 min	1-Monolinoleoylglycerol trimethylsilyl ether	0.46	$C_{24}H_{46}O_4Si$	428.7	Anti-inflammatory, Antioxidant

26.166 min	Glutaric acid, hexyl 3-methylbut-3-enyl ester	0.32	$C_{16}H_{28}O_4$	284.39	
26.390 min	Methadone N-oxide	0.15	$C_{21}H_{27}NO_2$	325.4	Activities at opioid receptors
26.584 min	Undecane, 5,6-dimethyl-	0.19	$C_{13}H_{28}$	184.36	
27.357 min	9,17-Octadecadienal, (Z)-	0.45	$C_{18}H_{32}O$	264.4	Antioxidant
28.833 min	trans-Farnesol	0.16	$C_{15}H_{26}O$	222.37	Antimicrobial, Anti-inflammatory
28.976 min	1-Monolinoleoylglycerol trimethylsilyl ether	0.18	$C_{24}H_{46}O_4Si$	426.7	Anti-inflammatory, Antioxidant

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DISCUSSION

Trichoderma spp. and *Streptomyces* spp. are known as biocontrol agents of fungal plant pathogens and have been recognized as a potential source of bioactive metabolites **(Tchameni *et al.*, 2017; Boudjeko *et al.*, 2017)**. Furthermore, their interaction and beneficial activity on plant production are highlighted when they act as part of a consortium, mainly as mixtures of different species of plant-growth promoting bacteria under normal and diverse stress conditions **(Santoyo *et al.*, 2021)**. In the present study, the potential of *Trichoderma* sp. *P3*, *Streptomyces cameroonensis* and their consortium was evaluated, and their extracts were used to develop formulations for the potential control of potato wilt disease caused by *Ralstonia solanacearum*.

In this study, the compatibility of *Trichoderma P3* with *Streptomyces cameroonensis* was confirmed by observing the growth of the mycelium and spores of the two microorganisms after 30 days of dual culture on PDA. According to the R1 (complete colonisation of medium by *Trichoderma*) to R5 (complete inhibition of *Trichoderma*) rating scale of **Bell *et al.* (1982)**, *Trichoderma P3* has the R2 rate with *Streptomyces cameroonensis* because it colonizes at least two-thirds of the medium surface. Moreover, *Trichoderma P3* and its coculture with *Streptomyces cameroonensis* have an R3 rate on the pathogens *Fusarium oxysporum* and *Phytophthora infestans*. However, an R4 rate was observed with *Trichoderma P3* and its coculture with *Streptomyces cameroonensis* against *Pythium myriotylum*. These results are less significant than those observed by **Bedine Boat *et al.* (2019)** who found an R1 rate by confronting *Trichoderma* It-13, It-21, It-58, It-62, P-8 and P-11 isolates with *F. oxysporum, F. solani, M. phaseolina, and P. ultimum.*

An alternative to synthetic fungicides is the use of biocontrol agents and/or their metabolites **(Stracquadanio *et al.*, 2020)**. In this study, the extraction of metabolites produced by *Trichoderma P3*, *Streptomyces cameroonensis,* and their coculture showed that the ethyl acetate extract is better than the hexane extract to control the growth of phytopathogens. These results could be explained by the capacity of ethyl acetate to extract diverse classes of metabolites as compared to hexane **(Stracquadanio *et al.*, 2020)**. However, other solvents such as methanol could be used to have increased class of metabolites.

The three-factor simplex design used in this study shows that the cubic model is best to predict the stability and activity of ethyl acetate extracts of *Trichoderma P3* and its coculture with *Streptomyces cameroonensis.* These results are different from those of **Foka *et al.* (2023),** who showed that, by using a four-factor design having essential oils and aqueous plant extracts plus Tween80, the linear model was best to predict the stability and activity against *Phytophthora infestans*. However, Tween80 has the same positive effect on the stability and negative effect on the activity model in the two studies.

The characterisation of these formulations based on the extract of *Trichoderma P3* and the extract of its coculture with *Streptomyces cameroonensis* showed that droplets have micro-sizes and are stable thermodynamically. This could be explained by the high concentration of Tween80, which aids in dispersing the oil droplets in both formulations, and the low concentration of oiled extracts relative to those of water **(Bhattacharya and Dixit 2015; Foka *et al.*, 2023)**.

The two bioformulations have the same minimum inhibitory concentration (MIC) and exhibit no significant difference when applied *in vitro* against *Ralstonia solanacearum*. Moreover, the formulations have a lytic effect on the bacteria cell wall and an inhibitory effect on the ATPase-H^+ pumps of the bacteria. Compared to *Trichoderma P3* alone, two modes of action were more significant for the coculture. This result corroborated with that of **Khethr *et al.* (2008),** who obtained a positive antibacterial value with butanolic extracts of *Trichoderma* sp.

Plant defence can generally be induced more effectively and efficiently by microbial consortia than by a single microbial inoculant. There is an ample opportunity to harness these mechanisms for controlling yield and diminishing diseases in an associated crop **(Sonker *et al.*, 2014)**. These new approaches of plant microbiome engineering consist of adding effective bioinoculants to induce new structured biological networks in diverse soil types. This promotes the recovery of beneficial microbial groups that are positively linked to soil fertility in both optimal conditions and under different types of biotic and abiotic stress **(Woo & Pepe, 2018)**. In the present study, the effects of the pretreatment of potato leaves with the bioformulation based on the *Trichoderma P3* extract and another based on the extract of its coculture with *Streptomyces cameroonensis* shows a reduction in disease severity. This may be explained by the bactericidal effect of the formulations as demonstrated in the present study and as previously demonstrated by **Khethr *et al.* (2008)** using *Trichoderma* extracts on bacteria.

Microorganism consortia are used to promote plant development or chemicals degradation **(Muhae-Ud-Din *et al.*, 2018; Santoyo *et al.*, 2021)**. The GC-MS analysis of the extracts in the present study and their coculture extracts shows the induction of new compounds in the consortium in comparison with those of *Trichoderma P3* alone or *Streptomyces cameroonensis* alone. There was also the absence of some compounds in the consortium extract, which was present in the extract of *Trichoderma P3* alone or *Streptomyces cameroonensis* alone. These observations can be explained by the capacity of *Trichoderma P3* and/or *Streptomyces cameroonensis* to transform the product of some pathways in the metabolism of the other microorganisms. This observation is supported by **Stracquadanio *et al.* (2020)** who demonstrated that the production and the class of metabolites produced by *Trichoderma* are influenced by the presence and type of other microorganisms.

Amongst the compounds induced by the coculture, there is 4-Imidazolidinone, 5-(phenylmethyl)-2-thioxo- which is an alkaloid that can have antimicrobial property and can be an inhibitor of H2 histamine receptors **(Rani *et al.*, 2015).** We also have 9-Octadecenoic acid (Z)-, methyl ester a methyl oleate which can reduce bacterial growth *in vitro* **(Padmini *et al.*, 2020)**. In the present study, among the volatile compounds, there were anti microbial compounds such as 3-Octadecanone with an activity on pathogens **(Ibrahem *et al.*, 2020; Rani *et al.*, 2023)** which can explain the lytic effect on cell wall integrity of the two formulations. Present were 40 compounds induced (present in the consortium and absent in the single cultures) with various properties like antibacterial, antimicrobial, antioxidant, antidiabetic, neuroprotective and anticancer activities. This may be explained by the presence of sub lethal compounds produced by one organism on the other which may enable it to produce another compound as demonstrated by **Khan *et al.* (2024)**.

CONCLUSION

This preliminary study is an evaluation of the potential compatibility and antibacterial activity of the coculture of *Trichoderma P3* and *Streptomyces cameroonensis* against *Ralstonia solanacearum.*

Streptomyces cameroonensis and *Trichoderma P3* were found to be compatible *in vitro*. The determination of *Streptomyces cameroonensis, Trichoderma P3,* and their coculture extracts MIC on *Ralstonia solanacearum* (3.33; 0.58; and 0.83 g/L respectively) permitted to develop the most active formulations based on *Trichoderma P3,* and their coculture with *Streptomyces cameroonensis*. The formulations acted on *Ralstonia solanacearum* by cell wall disruption and ATPase-H+ pump activity inhibition. In addition, the GC-MS analysis of the *Trichoderma P3*, coculture, and *Streptomyces cameroonensis* extracts revealed the presence of potential bioactive metabolites among which those induced in their coculture such as butyl citrate, and 4-Imidazolidinone, 5-(phenylmethyl)-2-thioxo having antimicrobial activities. However, the formulation did not significantly reduce disease severity on the detached potato leaves.

These results suggest that the coculture of *Trichoderma P3* and Streptomyces *cameroonensis* may be a potential environmentally friendly control agent for potato bacterial wilt caused by *Ralstonia solanacearum.* The next approaches would be to carry out plant assays in the greenhouse using their coculture and to identify metabolites present in the formulations responsible for antibacterial activity.

Statements and Declarations

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