

Improving the Microbiological Quality of Substrates and Biological Control Strategies to Optimize First-Generation Greenhouse Potato Seed Production in West Cameroon

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ABSTRACT

Potato production is on the increase in tropical regions, but yields remain low, generally between 3 and 11 t/ha, due to poor seed quality and phytosanitary pressures. First-generation seeds are a promising solution for improving productivity, but they require pathogen-free organic growing substrates (soil, compost, and sand mixtures) to guarantee plant health. The aim of this study, carried out from January to June 2022 in Bayangam, Cameroon, was to assess the microbiological quality of greenhouse substrates and propose biological control strategies. Analyses carried out before and after sterilization revealed high initial contamination of the irrigation water, with 193 CFU/mL total coliforms, making it unfit for use. Bacterial loads, including *Streptococcus* spp. and *Shigella* spp., as well as fungal populations such as *Fusarium* spp., were significantly reduced after sterilization. Pathogenicity tests identified *Fusarium* sp. as the most virulent, causing lesions of 7.66 cm² on potato leaves after 7 days. Furthermore, aqueous extracts of *Tephrosia vogelii* showed high antifungal activity, with inhibition rates reaching 93.71% against *Fusarium subglutinans*. These results demonstrate the importance of rigorous control of the microbiological quality of substrates and confirm that sterilization, combined with the use of botanical extracts, represents a sustainable alternative to chemical pesticides, thus contributing to healthy seed production and food security in Cameroon.

Keywords: Potatoes, Microbiological quality, Sterilization, Pathogenicity, West Cameroon.

RÉSUMÉ

La production de pomme de terre connaît un essor dans les régions tropicales ; néanmoins, les rendements demeurent faibles, s'établissant généralement entre 3 et 11 t/ha, en raison de la piètre qualité des semences et des pressions phytosanitaires. Les semences de première génération constituent une solution prometteuse pour accroître la productivité, mais elles exigent des substrats organiques de culture (mélanges de sol, compost et sable) exempts de germes pathogènes afin de garantir la santé des plants. L'objectif de cette étude, menée de janvier à juin 2022 à Bayangam (Cameroun), était d'évaluer la qualité microbiologique de substrats sous serre et de proposer des stratégies de lutte biologique. Les analyses réalisées avant et après stérilisation ont révélé une forte contamination initiale de l'eau d'irrigation, affichant 193 UFC/mL de coliformes totaux, la rendant ainsi impropre à la consommation. Les charges bactériennes, incluant *Streptococcus* spp. et *Shigella* spp., ainsi que les populations fongiques telles que *Fusarium* spp., ont été significativement réduites à la suite

de la stérilisation. Les tests de pathogénicité ont permis d'identifier *Fusarium* sp. comme le germe le plus virulent, occasionnant des lésions de 7,66 cm² sur les feuilles de pomme de terre au terme de 7 jours. En outre, les extraits aqueux de *Tephrosia vogelii* ont manifesté une activité antifongique élevée, avec des taux d'inhibition atteignant 93,71 % à l'encontre de *Fusarium subglutinans*. Ces résultats démontrent l'importance d'un contrôle rigoureux de la qualité microbiologique des substrats et confirment que la stérilisation, conjuguée à l'emploi d'extraits botaniques, représente une alternative durable aux pesticides chimiques, contribuant ainsi à une production semencière saine et à la sécurité alimentaire au Cameroun.

Mots-clés : Pomme de terre, Qualité microbiologique, Stérilisation, Pathogénicité, Ouest-Cameroun.

1. INTRODUCTION

Potato production in tropical areas is increasing; however, yields remain low, fluctuating between 3 and 11 t/ha (Peter 2019). This crop, which is vital for food security, faces several constraints, particularly the quality of the seeds used (Karanja et al. 2014). First-generation seeds (FGS), derived from rooted apical cuttings and mini-tubers, are a promising option for overcoming these difficulties (Rejeb & El 2007). Although alternative soilless systems like hydroponics or chemical soil fumigation exist, their adoption is severely constrained by high operational costs, technical complexity, and environmental hazards. Consequently, greenhouse production relies heavily on organic growing substrates (soil, compost, and sand mixtures), which require stringent sanitization to guarantee plant vigor and productivity.

Worldwide, the potato is one of the most widely consumed staple foods, with production estimated at around 383 million tons in 2024 (FAO 2024). In Africa, potato productivity remains constrained, with average yields of approximately 15.8 t/ha, well below potential capacity (FAOSTAT 2023). There is production potential in Cameroon, with 354,404 tons harvested in 2020 (Joseph, 2023). However, this production is severely constrained by limited access to high-quality seeds and high-performance cultivation practices. In addition, there are difficulties linked to the microbiological quality of the substrates used in greenhouses, which weaken crops and encourage the spread of disease, leading to a considerable reduction in yields (Mohamed 2019, Shah et al. 2021; Ntangmo et al., 2025).

The instability of potato yields is mainly attributed to the shortage of certified seeds and the risk of microbial contamination of greenhouse growing substrates (GIZ 2019, Sawicka et al. 2021). The presence of pathogens in these substrates can cause infections as early as the first generation of plants, compromising their vigor and growth (Bradford et al. 2013). The agronomic consequences are significant, ranging from reduced yields to increased food insecurity in regions where potatoes are an essential staple (Alo 2021, McGuire & Sperling 2013, Devaux et al. 2014, Temateu et al., 2026). In addition, the deterioration in the sanitary state of crops leads to increased recourse to phytosanitary treatments, which adds to production costs and calls into question the sustainability of farming systems (Gullino et al. 2022; Alo, 2021).

Potato cultivation also plays a major economic role for many producers in Cameroon (Chiankem 2022). Thus, improving yields could have a direct impact on food security and local economic development. Moreover, quality seed production is an essential lever for supporting agricultural development and strengthening the resilience of food systems to climatic and economic hazards (McGuire & Sperling 2013). Therefore, we aimed to contribute to the qualitative and quantitative improvement of first-generation potato seeds produced in greenhouses by assessing the microbiological quality of growing substrates and proposing biological control strategies to mitigate the risk of contamination. Through in-depth microbiological analyses, we provide essential data to promote sustainable solutions and thus strengthen first-generation potato seed production in Cameroon.

2. MATERIALS AND METHODS

2.1. Setting and study period

The study was conducted from January to June 2022 in a greenhouse set up in Bayangam, a locality in the Koung-Khi department in the West region of Cameroon, about 25 km from Bafoussam. The site is at an altitude of 1900 m, and its geographical coordinates are N 05° 16'34,01" latitude and E 010° 25'92,8" longitude. Bayangam was chosen for this study for several key reasons. First, its altitude provides a temperate climate and agroecological conditions favorable to potato cultivation. Second, its proximity to Bafoussam makes it easy to benefit from the infrastructure and resources.

Samples were taken before and during the production cycle at the greenhouse's water supply point. Samples were stored in clean, sterile containers and sent to the laboratory within 24 h for microbiological analysis.

Microorganisms present in the water were isolated using the decimal dilution method described by Agbodjato et al. (2017). This procedure involved making a series of dilutions of the stock solution, from 10⁻¹ to 10⁻⁷, and then inoculating 100 µL of the 10⁻⁵ and 10⁻⁷ dilutions on selective media. Coliforms were identified on MacConkey agar and incubated at 37°C for total coliforms and 44°C for fecal coliforms. Results were expressed as colony-forming units (CFU) per 100 mL of water. Streptococcus was detected in Rothe broth incubated at 44°C for 24 h, followed by Gram staining. Salmonella spp. and Shigella spp. were enriched in selenite broth at 37°C, then plated on Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C. Staphylococcus spp. were detected after enrichment in trypticase soy broth at 37°C, then plated onto salted mannitol agar, also incubated at 37°C. Finally, Vibrio spp. were isolated after enrichment in Thiosulfate-Citrate-Bile Salts (TCBS) broth and incubated at 30°C. The presence of yellow colonies on TCBS agar confirmed their identification.

2.2. Isolation of bacteria in substrates before and after sterilization

Samples were taken before and after substrate sterilization, using the random sampling method described by Razavi et al. (2007). For each heap, 10 to 15 core sub-samples were collected and pooled into a composite sample. A 10 g analytical unit was homogenized in 90 mL of sterile physiological saline (0.9% NaCl) to obtain a 10⁻¹ suspension. Serial decimal dilutions were carried out from 10⁻¹ to 10⁻⁷. Aliquots of 100 µL from dilutions 10⁻⁵ and 10⁻⁷ were spread-plated onto Plate Count Agar (PCA) and Nutrient Agar in triplicate. Plates were incubated at 37°C for 48 h. Total bacterial enumerations were expressed as CFU/g of dry substrate. Pure bacterial cultures were obtained by streak-purification.

2.3. Identification and quantification of bacteria in substrates before and after sterilization

The Gram stain, morphological characterization of cells, catalase test, motility on semisolid agar, citrate test on Simmons medium, and urease test on urea-indole medium were performed for preliminary phenotypic characterization (Murray et al. 2003, Pacheco et al. 2023). Taxonomic confirmation was then established using the API 20 biochemical identification gallery, generating a numerical profile (7-digit profile code) analyzed via the dedicated knowledge base. Since molecular analysis by 16S rRNA gene sequencing was not performed in this study, it remains a prospect for in-depth genomic characterization.

2.4. Isolation and Identification of Fungi in Substrates Before and After Sterilization

The fungi present in substrates were isolated using the decimal dilutions method described by Agbodjato et al. (2017). Successive dilutions were prepared up to 10⁻⁷, and then 100 µL of the 10⁻⁵ and 10⁻⁷ dilutions were seeded onto Potato Dextrose Agar (PDA) culture medium. For each dilution, three Petri dishes were used as replicates. Fungal colonies grew in the substrates after 7 days of incubation at room temperature. Each colony obtained was then cut and replated several times in new Petri dishes containing PDA until purified strains were obtained. Purified fungi were identified using light microscopy, based on their morphological and cultural characteristics, in accordance with the identification keys and descriptions proposed by Shaw (2022) and Dongmo et al. (2017).

2.5. Pathogenicity assessment of fungi

The aim of the pathogenicity test was to assess the ability of the fungi most commonly found in substrates after sterilization to induce disease in potatoes. Fresh, healthy leaves were taken from young plants and disinfected with 70% alcohol. They were then lightly incised with a sterile scalpel to promote fungal penetration, before being inoculated with 5 mm (diameter) mycelial explants (Chelahi & Benguerba 2021). The inoculated leaves were laid out on moist absorbent paper and incubated at room temperature and a relative humidity of >90% for 7 days. Observations focused on the virulence of the fungi, i.e., their ability to cause characteristic symptoms on potato leaves. Virulence was assessed by measuring the diameter of necrotic lesions on days 3, 5, and 7 after inoculation, then compared with values obtained from non-inoculated controls (Biessy et al. 2024).

2.6. Study of the in Vitro Effects of Phytosanitary Treatments Based on *Tephrosia vogelii* Extract on the Fungi *Fusarium subglutinans*, *Alternaria solani*, and *Fusarium* sp.

To obtain *Tephrosia vogelii* extract, we harvested fresh leaves and dried them in the shade to preserve the active compounds. Once the leaves were completely dry, we ground them to a fine powder. Extraction was carried out using ethanol for the ethanolic extract and water for the aqueous extract, by mixing 100 g of powder with 1 L of solvent. After macerating for 24 h at room temperature, the mixture was filtered, and the solvent was evaporated to obtain a concentrated extract (Teryila 2015). The crude extracts were weighed to determine the extraction yield. The extraction yield (Rdt%) was calculated according to the following formula:

Rdt (%) = $M1/M0 \times 100$ (Salem 2009), where M0 is the mass of the initial plant material, and M1 is the mass of the extract.

In vitro, the antifungal activity of the extracts was evaluated at concentrations of 5, 7.5, and 10 mg/mL for aqueous extracts, and 15, 20, and 25 mg/mL for ethanolic extracts. The plant extracts were incorporated into molten PDA medium at approximately 45°C under aseptic conditions, thoroughly mixed, and poured into sterile Petri dishes. Isolated phytopathogenic fungal mycelial explants 5 mm in diameter were cut using a sterile cork borer from pure cultures and placed in the center of each Petri dish. Three plates were seeded per treatment, each constituting a replicate.

Samples were incubated at $23 \pm 1^\circ\text{C}$ for one week. Daily measurements of the radial growth diameter of each cultured explant were taken continuously until the mycelium filled the control plates. Negative control plates contained the culture medium without extract, while positive control plates were supplemented with a commercial synthetic fungicide, Bonsoin (active ingredients: 30% Chlorothalonil + 6% Cymoxanil WP), at the recommended field concentration. Radial growth inhibition of the pathogen was expressed as a percentage of inhibition (PI) and calculated according to the formula of Vincent (1947):

$$PI = ((A-B)/A) \times 100$$

Where A is the average mycelial diameter (mm) of the negative control, and B is the average mycelial diameter (mm) in the presence of the extract or the positive control fungicide. Regarding the inhibition values, the reduction in mycelial growth ranged from 2.50 ± 0.71 mm to 0.50 ± 0.71 mm.

2.7. Statistical data analysis

Statistical analysis of the data was carried out using Excel 2016 and the Statistical Package for the Social Sciences (SPSS). Experimental data were first entered and organized in an Excel spreadsheet, enabling the verification of their integrity and the identification of missing values. An analysis of variance was performed with SPSS to compare the means of the different experimental groups, followed by a Duncan test to separate means. Significance was set at the 5% probability threshold ($p < 0.05$). Results were presented in tabular and graphical forms, including means, standard deviations, and p-values, to facilitate visualization of trends.

3. RESULTS

3.1. Microbiological characteristics of irrigation water

The irrigation water at the Bayangam greenhouse showed significant microbiological contamination, with a total coliform load of 193 CFU/mL. According to World Health Organization (WHO) recommendations, this water was deemed unfit for human consumption. It was also a source of contamination for the greenhouse substrate and Vitro plants (Table 1).

Table 1: Characteristics of irrigation water used at the Bayangam greenhouse

Appearance	Color	Odor				
Clear	Colorless	Acre				
Number of bacteria in 100 ml of water						
Total Coliforms	<i>E. coli</i>	<i>Streptococcus spp</i>	<i>Salmonella spp</i>	<i>Shigella spp</i>	<i>Staphylococcus spp</i>	<i>Vibrio spp</i>
193	90	40	50	5	5	3

3.2. Type and number of bacteria present in substrates before and after sterilization

Table 2 presents the bacterial load of substrates before and after sterilization, highlighting significant differences for several bacterial types. The results were particularly revealing for *Streptococcus* spp., whose load significantly decreased from 2.50 ± 0.71 to 0.50 ± 0.71 ($p = 0.02$). *Shigella* spp. also showed a significant decrease, from 18.50 ± 7.77 to 0.50 ± 0.71 ($p = 0.021$). Furthermore, *Staphylococcus* spp. counts dropped significantly from 50 ± 0.71 to 0.00 ± 0.00 ($p < 0.001$). Finally, *Vibrio* spp. counts significantly decreased, from 206.00 ± 96.167 to 35.00 ± 19.49 ($p = 0.004$). Conversely, *E. coli* counts increased post-sterilization, rising from 0.00 ± 0.00 to 2.00 ± 1.41 ; however, this change was not significant ($p = 0.107$). This trend can be attributed to

the creation of a "biological vacuum" following the drastic reduction of competing microbial populations (*Staphylococcus* spp. and *Vibrio* spp.), which allows opportunistic bacteria to proliferate rapidly if minor cross-contamination occurs during substrate handling or cooling under greenhouse conditions.

Table 2: Bacterial load before and after sterilization

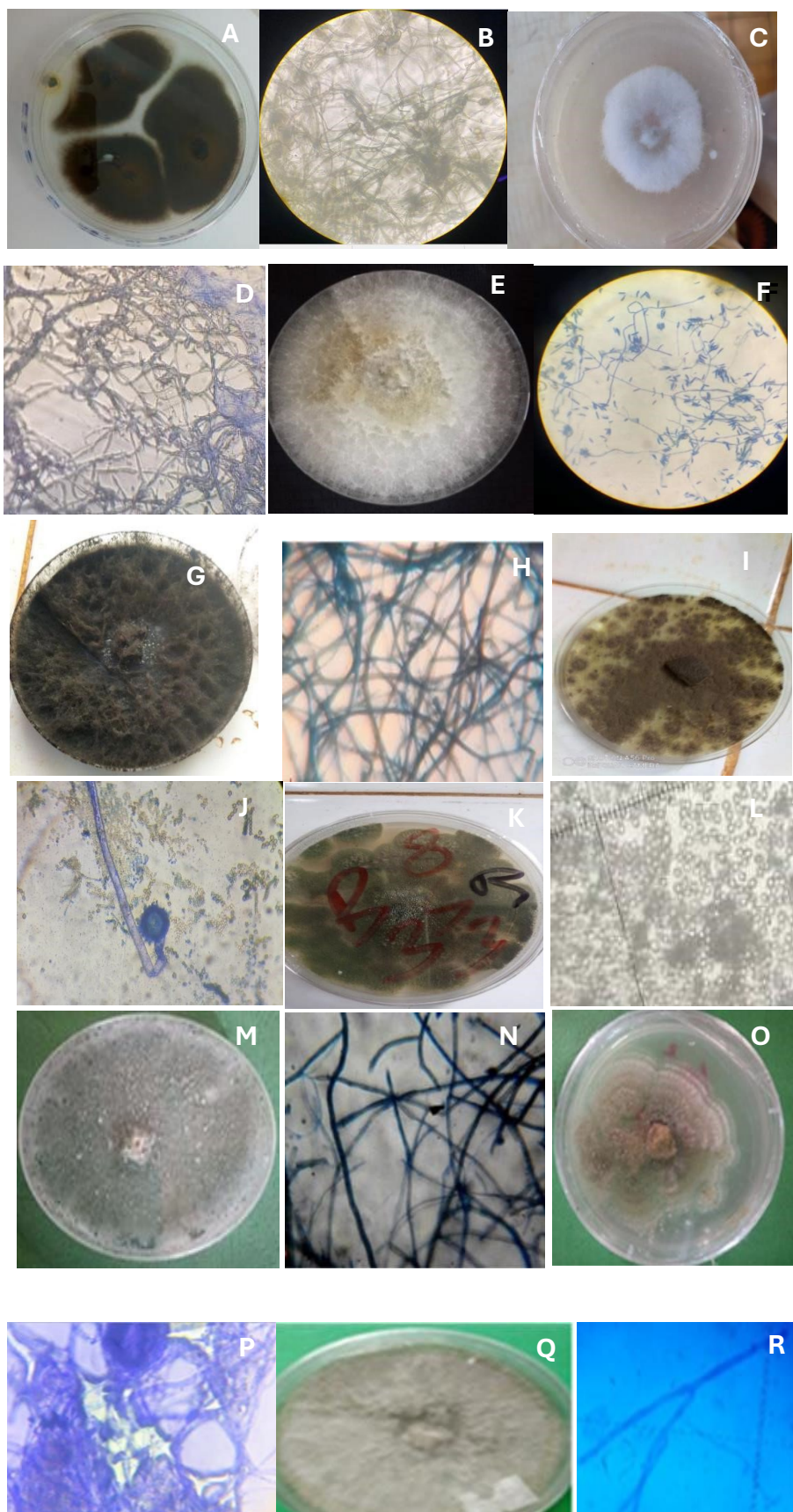
Type of bacteria	Bacterial load		P-value
	Before sterilization	After sterilization	
<i>Total coliforms</i>	00,00±00,00	0,00±0,00	0.479
<i>E. coli</i>	0,00±0,00	2,00±1,41	0.107
<i>Streptococcus</i> spp.	2,50±0,71	0,50±0,71	0.02*
<i>Shigella</i> spp.	18.50±7,77	0,50±0,71	0.021*
<i>Staphylococcus</i> spp.	5,50±0,71	0,00±0,00	< 0. 001***
<i>Vibrio</i> spp.	206,00±96,167	35,00±19,49	0,004**

P-value: Significance Level; $p < 0.001$ ***; $P < 0.01$ **; $P < 0.05$ *.

3.3 Type of fungi present in substrates before and after sterilization

Analysis of the fungal isolates present in the substrates before and after sterilization identified several species, each with distinct macroscopic and microscopic characteristics. *Alternaria solani* was characterized, in pure culture, by a blackish mycelium with slow, uneven development on PDA medium, and by numerous, crisscrossing spindle-shaped conidia (Figures 1A and 1B). *Fusarium subglutinans* showed a whitish mycelium developing very rapidly on PDA, with numerous, curved, intertwined filaments (Figures 1C and 1D). *Fusarium* sp. was distinguished by a cottony white mycelium composed of septate hyaline filaments, branched at acute and right angles, and numerous spores scattered between the filaments (Figures 1E and 1F). *Cercospora* sp. initially formed cottony-white colonies becoming progressively blackish, with slow growth; its mycelium produced short, compact conidiophores bearing long, septate, slightly curved conidia (Figures 1G and 1H). *Aspergillus niger* could be recognized by its black mycelium and spherical, sometimes ribbed conidia, surrounded by *Aspergillus biseria* heads and *phialid metules* (Figures 1I and 1J). *Aspergillus fumigatus* presented slow-growing, powdery green colonies, with numerous spherical spores observable under the microscope (Figures 1K and 1L). *Trichoderma* spp. were distinguished by their spaced green colonies and rapid growth on PDA, with mycelial filaments interspersed with spores (Figures 1M and 1N). *Aspergillus terreus* developed a brownish mycelium at maturity, with flat colonies forming short hyaline and septate filaments, and a characteristic aspergillate head (Figures 1O and 1P). Finally, *Rhizoctonia solani* appeared as radiating greyish mycelial colonies, growing radially very rapidly on PDA, with light-brown septate mycelium slightly constricted at the septa and devoid of spores (Figures 1Q and 1R).

3.4. Number of fungi present in substrates before and after sterilization. Table 3 shows the fungal load of substrates before and after sterilization, highlighting significant differences for several species. *Alternaria solani* load decreased from 124.12 ± 15.50 to 90.34 ± 13.70 ($p = 0.03$). *Fusarium subglutinans* showed a highly significant decrease, from 724.50 ± 11.23 to 100.41 ± 19.71 ($p = 0.001$), while *Fusarium* sp. also showed a highly significant reduction, from 607.2 ± 21.34 to 64.65 ± 32.35 ($p < 0.001$). *Cercospora* sp. exhibited a significant decrease in fungal load from 17.50 ± 3.54 to 12.50 ± 3.54 ($p = 0.04$). That of *Aspergillus niger* experienced a non-significant decrease ($p = 0.03$). In contrast, *Aspergillus fumigatus* underwent a drastic reduction in fungal load, from 963.50 ± 122.32 to 63.50 ± 14.84 , with a highly significant p-value ($p < 0.001$). *Trichoderma* sp. fungal load decreased significantly, from 82.00 ± 24.00 to 18.50 ± 4.95 ($p = 0.01$), as did that of *Aspergillus terreus*, from 135.00 ± 22.63 to 10.00 ± 2.83 ($p = 0.001$). That of *Alternaria alternata* decreased slightly, from 355.23 ± 109.5 to 289.75 ± 84.01 . Finally, *Rhizoctonia solani* showed a significant reduction in fungal load from 64.50 ± 20.51 to 9.50 ± 0.70 ($p = 0.012$).



A and B, *Alternaria solani*; C and D, *Fusarium subglutinans*; E and F, *Fusarium* sp.; G and H, *Cercospora* sp.; I and J, *Aspergillus niger*; K and L, *Aspergillus fumigatus*; M and N, *Trichoderma* sp.; O and P, *Aspergillus terreus*; Q and R, *Rhizoctonia solani*.

Figure 1: Fungal colonies on PDA medium and microscopic appearance of mycelium and spores of fungi isolated on substrates before and after sterilization.

Table 3: Fungal load of substrates before and after sterilization

Mushrooms	Fungal load		P-value
	Before sterilization	After sterilization	
<i>Alternaria solani</i>	124,12±15,50	90,34±13,7	0.03*
<i>Fusarium subglutinans</i>	724,5±11,23	100,41±19,71	0.001*
<i>Fusarium sp.</i>	607,2±21,34	64,65±32,35	0.000***
<i>Cercospora spp.</i>	17,50±3,54	12,5±3,54	0.04*
<i>Aspergillus niger</i>	4,00±1,	3,00±3,24	0.03
<i>Aspergillus fumigatus</i>	963,50±122,32	63,50±14,84	0.000***
<i>Aspergillus terreus</i>	135,00±22,63	10,00±2,83	0.001**
<i>Trichoderma sp.</i>	82,00±24,00	18,50±4,95	0.01*
<i>Rhizoctonia solani</i>	64,50±20,51	9,50±0,70	0.012*

P-value: Significance Level; $p < 0.001$ ***; $P < 0.01$ **; $P < 0.05$ *.



C1, *Aspergillus fumigatus*; C2, *Alternaria solani*; C3, *Fusarium sp.*; C4, *Fusarium subglutinans*; T, Control. A, Fresh leaves just after receiving mycelial explant; B, fresh leaves 3 days after seeding; C, fresh leaves 5 days after seeding; D, fresh leaves 7 days after seeding; E, control 3 days after seeding; F, control 5 days after seeding; G, control 7 days after seeding.

Figure 2: Illustration of the pathogenicity test carried out with fungi whose fungal load was found to be very high after sterilization.

3.5. Results of pathogenicity tests with fungi

Figure 2 and Table 3 examine the effect of different fungi on lesion area measured in square centimeters. The data show a significant variation in lesion area depending on the fungus tested. Figure 2 illustrates the pathogenicity test conducted on different fungal species, particularly those with a high fungal load after sterilization. These include: *Aspergillus fumigatus*; *Alternaria solani*; *Fusarium sp.*; and *Fusarium subglutinans*. Observations were made on fresh leaves at various times after seeding.

Table 3 presents the evolution of lesion area induced by the different fungal strains at 3, 5, and 7 days post-inoculation (DPI), along with the overall mean lesion surface and significance. The control maintained a lesion area of $0.00 \pm 0.00 \text{ cm}^2$ at 3 DPI, $0.50 \pm 0.10 \text{ cm}^2$ at 5 DPI, and $1.00 \pm 0.00 \text{ cm}^2$ at 7 DPI, yielding an overall mean of $0.50 \pm 0.11 \text{ cm}^2$. For *Aspergillus fumigatus*, lesion area increased from $1.20 \pm 0.45 \text{ cm}^2$ at 3 DPI to $3.10 \pm 1.20 \text{ cm}^2$ at 5 DPI, reaching $5.50 \pm 3.26 \text{ cm}^2$ at 7 DPI, with an overall mean of $2.75 \pm 2.69 \text{ cm}^2$ ($p = 0.031$). *Alternaria solani* induced lesions of $2.10 \pm 0.80 \text{ cm}^2$, $4.80 \pm 1.50 \text{ cm}^2$, and $6.50 \pm 2.00 \text{ cm}^2$ at 3, 5, and 7 DPI, respectively, resulting in an overall mean of $4.69 \pm 2.80 \text{ cm}^2$ ($p = 0.012$). *Fusarium subglutinans* produced lesion surfaces of $2.05 \pm 0.60 \text{ cm}^2$ at 3 DPI, $4.40 \pm 1.10 \text{ cm}^2$ at 5 DPI, and $6.08 \pm 2.09 \text{ cm}^2$ at 7 DPI, with an overall mean of $4.66 \pm 1.98 \text{ cm}^2$ ($p = 0.008$). Finally, *Fusarium sp.* recorded lesion areas of $3.10 \pm 0.90 \text{ cm}^2$, $6.00 \pm 1.80 \text{ cm}^2$, and $7.66 \pm 1.44 \text{ cm}^2$ at 3, 5, and 7 DPI, resulting in the highest overall mean of $6.11 \pm 2.54 \text{ cm}^2$ ($p < 0.001$).

Table 3: Dynamic progression and overall mean of lesion area (cm^2) induced by fungal strains at 3, 5, and 7 DPI

Fungal species	3 DPI (cm^2)	5 DPI (cm^2)	7 DPI (cm^2)	Overall Mean (cm^2)	P-value
Control	0.00 ± 0.00	0.50 ± 0.10	1.00 ± 0.00	0.50 ± 0.11	-
<i>Aspergillus fumigatus</i>	1.20 ± 0.45	3.10 ± 1.20	5.50 ± 3.26	3.27 ± 1.69	0.031*
<i>Alternaria solani</i>	2.10 ± 0.80	4.80 ± 1.50	6.50 ± 2.00	4.46 ± 2.80	0.012*
<i>Fusarium subglutinans</i>	2.05 ± 0.60	4.40 ± 1.10	6.08 ± 2.09	4.17 ± 1.98	0.008**
<i>Fusarium sp.</i>	3.10 ± 0.90	6.00 ± 1.80	7.66 ± 1.44	5.59 ± 2.54	< 0.001***

P-value, Significance level relative to control; $p < 0.001$; $p < 0.01$; $p < 0.05$.

3.6. Effects of *in vitro* phytosanitary treatments of *Tephrosia vogelii* extract on the fungi *Fusarium subglutinans*, *Alternaria solani*, and *Fusarium sp.*

Table 4 presents the effect of different concentrations of aqueous (15, 20, and 25 mg/mL) and ethanolic extracts (5, 7.5, and 10 mg/mL) on the PIs of *Fusarium subglutinans*, *Alternaria solani*, and *Fusarium sp.* For aqueous extracts, increasing the concentration from 15 to 25 mg/mL increased the inhibition percentage, reaching $93.71 \pm 5.67\%$ for *Fusarium subglutinans*, $83.09 \pm 7.91\%$ for *Alternaria solani*, and $92.86 \pm 9.03\%$ for *Fusarium sp.* at 25 mg/mL.

For ethanolic extracts tested at lower concentrations (5, 7.5, and 10 mg/mL), maximum inhibition for *Fusarium subglutinans* was observed at 5 mg/mL ($88.17 \pm 5.97\%$), whereas *Alternaria solani* and *Fusarium sp.* reached maximum inhibition rates at 7.5 mg/mL ($88.21 \pm 14.73\%$) and 10 mg/mL ($81.37 \pm 11.38\%$), respectively. These interspecific variations in antifungal susceptibility can be attributed to differences in fungal cell wall composition and membrane permeability, which govern the rate of intracellular uptake of bioactive lipophilic compounds (flavonoids, alkaloids, and polyphenols) extracted in ethanol. The inverse dose-response trend observed for *F. subglutinans*, where maximum efficacy occurred at the lowest tested concentration (5 mg/mL), may result from solubility limitations, self-aggregation of polyphenolic compounds at higher concentrations, or biphasic hormetic effects. Conversely, the progressive, concentration-dependent inhibition seen in *A. solani* and *Fusarium sp.* highlights a dose-threshold mechanism required to disrupt fungal cell membrane integrity and inhibit key enzymatic pathways involved in fungal growth. The positive control induced total inhibition ($100.00 \pm 0.00\%$) for *Alternaria solani* and *Fusarium sp.*, and $64.28 \pm 0.00\%$ for *Fusarium subglutinans*. The negative control exhibited baseline inhibition levels of $57.14 \pm 0.00\%$ for *Fusarium subglutinans*, $48.56 \pm 5.15\%$ for *Alternaria solani*, and $52.85 \pm 5.13\%$ for *Fusarium sp.*

Table 4: Effect of different doses of aqueous and ethanoic extracts on percentage fungal inhibition.

Concentration (mg/ml)	Percentage of inhibition (I in %)		
	<i>Fusarium subglutinans</i>	<i>Alternaria solani</i>	<i>Fusarium sp</i>
Aqueous extracts			
15	82,55±21.30 ^a	89,81±9.61 ^{ab}	84,81±4.20 ^{ab}
20	90,89±6,50 ^a	86,01±1,69 ^{ab}	77,30±9,31 ^{bc}
25	93,71±5,67 ^a	83,09±7,91 ^b	92,86±9,03 ^{ab}
Ethanolic extracts			
5	88.17±5.97 ^a	86,50±10.98 ^{ab}	66,26±19.58 ^c
7.5	79,87±13.59 ^a	88,21±14,73 ^{ab}	80,03±12,01 ^{bc}
10	85,71±13.64 ^a	84,83±6,80 ^b	81,37±11,38 ^{bc}
Control			
Positive	64,28±0.00 ^b	100,00±0.00 ^a	100, 00±0.00 ^a
Negative	57,14±0,00 ^b	48,56±5.15 ^c	52,85±5.13 ^c

4. DISCUSSION

The results of microbiological analyses showed that the irrigation water used in the Bayangam greenhouse contained numerous bacteria such as enterobacteria, fecal coliform *E. coli*, Salmonella, Shigella, Staphylococcus, and Vibrio spp., was highly unsuitable for human consumption, and was a source of substrate recontamination during the production cycle of apical cuttings and/or mini-tubers. These results corroborate those of Ombala (2013), who showed that when water is susceptible to surface water infiltration, it becomes polluted and unsuitable for micro-irrigation. The presence of these bacteria in the water would be linked to its point of capture (a surface water stream), where it is in direct contact with surface water.

Analysis of the microbiological quality of the substrates before and after sterilization revealed that certain Gram-positive bacteria, such as *Streptococcus spp.* and *Staphylococcus spp.*, as well as Gram-negative bacteria such as *Shigella spp.*, showed a marked decrease after sterilization. These results corroborate those of Feurhuber et al. (2024), who observed a total elimination of *Staphylococcus spp.* Explained by the simpler structure of their cell wall, which increases their sensitivity to heat treatment. On the other hand, *Streptococcus spp.* showed an 80% reduction, which is consistent with data from Busto et al. (2022), who showed that this type of bacteria undergoes reductions of the order of 80% when exposed to heat. This reduction could be explained by the fact that their cell wall, although resistant, is sufficiently heat-sensitive to cause significant cell destruction, as heat damages protein structures and membranes, compromising bacterial integrity and viability.

Analysis of the fungal quality of substrates before and after sterilization revealed differential effectiveness in sterilization. Fungi of the genus *Fusarium* showed significant reductions, with 86.1% for *Fusarium subglutinans* and 89.4% for *Fusarium sp.*, corroborating Hudaib (2024), who showed that sterilization efficacy varies by fungal species. This variation can be explained by the cell structure and spore resistance of each fungal species, some of which have more robust walls or protective mechanisms to better withstand sterilization conditions.

The results of the pathogenicity test on the predominant fungi after sterilization revealed distinct virulence profiles according to fungal species. The maximum lesion area, reaching $7.66 \pm 1.44 \text{ cm}^2$ at 7 DPI, was observed with *Fusarium sp.*, confirming its status as a major pathogen. These results corroborate those of Tomilova et al. (2021), who showed that potato leaves inoculated with entomopathogenic fungi could have a decreased concentration of photosynthetic pigments, leading to lesion formation. This aggressiveness is explained by *Fusarium sp.*'s ability to produce toxic metabolites that disrupt the plants' physiological processes, causing significant damage and reducing their overall health.

Finally, the results showed that the efficacy of the extracts varied greatly with concentration and extraction method. Aqueous extracts proved more effective than ethanolic extracts. The aqueous extracts achieved remarkable inhibition rates at a concentration of 25 mg/mL: 93.71% against *Fusarium subglutinans* and 92.86% against *Fusarium sp.*, in line with the work of Mlozi et al. (2020), who showed that *Tephrosia vogelii* leaf and root extracts have antimicrobial potential against various pathogenic fungi and bacteria, thus offering an alternative to chemical pesticides. This efficacy could be explained by the presence of water-soluble bioactive compounds, such as flavonoids and tannins, with antifungal and antibacterial properties, in aqueous extracts.

This study has some limitations that may influence the generalizability of its results. Since it was conducted only in the Bayangam locality, it may not be representative of other regions of Cameroon, where climatic conditions and soil characteristics differ. In addition, although the sampling method is rigorous, it may have introduced bias by failing to capture the natural variability of substrates and irrigation water. Our research also focused on specific bacterial and fungal species, neglecting an exhaustive assessment of all potentially present microorganisms, limiting a complete understanding of the microbiological landscape. Finally, pathogenicity tests have not accounted for interactions between different pathogens or multiple environmental stresses, which are crucial to plant health.

5. CONCLUSION

In this study, we highlighted the importance of the microbiological quality of substrates for greenhouse potato seed production in Cameroon. The results revealed significant contamination of irrigation water and substrates, highlighting the risks associated with the use of untreated resources. Sterilization proved effective in reducing bacterial and fungal loads, particularly for pathogens such as *Fusarium subglutinans* and *Fusarium sp.*, which showed high virulence in pathogenicity tests. Furthermore, *Tephrosia vogelii* extracts have shown potential as a biological alternative for controlling these pathogens, offering a sustainable approach to crop disease control. The results of this research provide a sound basis for developing improved agricultural practices aimed at enhancing food security and the resilience of potato production systems in the face of environmental challenges. Further research is recommended to explore microbiological interactions and optimize biological control strategies for healthy potato seed production in Cameroon.

ABBREVIATIONS

API 20: Analytical Profile Index 20 (biochemical identification strip system)
 C: Degree Celsius (unit of temperature measurement)
 CBS: Cameroon Biosciences Society
 CFU: Colony-Forming Unit
 cm: Centimeter (unit of length)
 cm²: Square centimeter (unit of surface area)
 cv.: Cultivar (cultivated variety)
 DOI: Digital Object Identifier
 DPI: Days Post-Inoculation
 E: East (geographic coordinate system)
 E. coli: Escherichia coli
 FAO: Food and Agriculture Organization of the United Nations
 FAOSTAT: Corporate Statistical Database of the Food and Agriculture Organization
 FGS: First-Generation Seeds
 g: Gram (unit of mass)
 GIZ: Deutsche Gesellschaft für Internationale Zusammenarbeit (German Corporation for International Cooperation)
 ha: Hectare (unit of area)
 IPC: International Potato Center
 IRAD: Institute of Agricultural Research for Development (Institut de Recherche Agricole pour le Développement)
 L: Liter (unit of volume)
 m: Meter (unit of elevation/altitude)
 mg: Milligram (unit of mass)
 mL: Milliliter (unit of volume)
 mm: Millimeter (unit of length)
 N: North (geographic coordinate system)
 NaCl: Sodium chloride (saline solution)
 p / P-value: Probability value (statistical level of significance)
 PCA: Plate Count Agar
 PDA: Potato Dextrose Agar
 PGPR: Plant Growth-Promoting Rhizobacteria
 PI: Percentage of Inhibition
 PROCISA: Project for the Promotion of Innovation Culture and Agricultural Structuring (Projet de Promotion de la Culture d'Innovation et de la

Structuration Agricole)

Rdt %: Extraction yield percentage (Rendement)

rRNA 16S: 16S Ribosomal Ribonucleic Acid

spp. / sp.: Species

SPSS: Statistical Package for the Social Sciences

t/ha: Tons per hectare

TCBS: Thiosulfate-Citrate-Bile Salts-Sucrose agar

UFC: Unité Formant Colonie (French equivalent of Colony-Forming Unit)

μL: Microliter (unit of volume)

WHO: World Health Organization

WP: Wettable Powder

XLD: Xylose Lysine Deoxycholate agar

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