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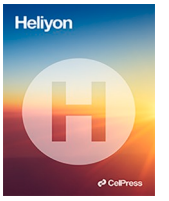

Antibacterial fibers impregnated with mycosynthetized AgNPs for control of *Pectobacterium carotovorum*

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ABSTRACT

Using biopolymers functionalized with antibacterial agents to manufacture active packaging is a clean alternative to mitigate food losses due to postharvest plant diseases. In this study, two mycosynthetized AgNPs impregnation methodologies on cotton (cationization and *in situ* biochemical reduction) were used to obtain the antibacterial fibers (A-AgNPs-C and A-AgNPs-IBR), which, in addition to being characterized by SEM-EDX, XRD, were evaluated as antibacterial materials. The cotton fibers showed growth inhibition of *Pectobacterium carotovorum* at 48 h. The reuse tests of these cotton fibers showed that the two types of fibers could have up to three successive uses without losing their effectiveness, regardless of the impregnation method used. Is important to highlight that the retention tests indicated that the AgNPs remain attached to the A-AgNPs-C and A-AgNPs-IBR fibers after several successive washes. Finally, the mycosynthesized AgNPs were also impregnated on fique fibers (Fique-AgNPs) by cationization to obtain little antibacterial sacks. Nanostructured materials that in *in vivo* tests on potatoes showed only 7.8 % of affectation, while the tubers stored in the traditional sacks had an affectation of 25 %. This immobilization of AgNPs in natural fibers will allow the development of a nanobiotechnological application in the storage and transport of potatoes, after performing some additional cytotoxicity tests to guarantee its safety.

1. Introduction

Given their high moisture content (75–90 %), many microorganisms proliferate on vegetables and fruits, affecting their production between harvest and consumption periods [1,2]. These postharvest losses of perishable foods in developing countries have been estimated at 30–50 % of production, representing millions of dollars for the food industry and millions of people to whom food does not reach [3,4]. Most postharvest diseases are attributed to the effect of various fungal genera such as *Alternaria*, *Aspergillus*, *Botrytis*, *Colletotrichum*, *Diplodia*, *Monilinia*, *Penicillium*, *Phomopsis*, *Rhizopus*, *Mucor*, *Sclerotinia*, and bacterial genera such as *Pseudomonas* sp., and *Pectobacterium* sp [1].

The *Pectobacterium carotovorum* is relevant for causing damage in multiple plant species, including potatoes (*Solanum tuberosum*),

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and at the postharvest level, it is the causal agent of soft tuber rot, a disease that generates significant economic losses [5–7]. Postharvest losses that can reach up to 60 % of production and occur anytime, from harvest to consumption. For this reason, *P. carotovorum* is one of the most severe problems in modern agriculture and the food industry worldwide [8], and since the potato is the most consumed vegetable in the world as a source of high-quality nutrients, it is imperative to develop strategies that protect this agricultural product once it is harvest [9,10]. Therefore, these strategies should include textile sacks, specifically fique (*Furcraea andina*) sacks [10,11], an innovative biotechnological application in the field of potatoes packaging, where antibacterial fique sacks can preserve the quality and extend the shelf life of potatoes while minimizing damage caused by bacteria such as *P. carotovorum* [4, 10–16]. This is because fique has cellulose as its main component, a material widely used in food packaging [15–18], whose linear homopolysaccharide structure, with many hydroxyl groups available for chemical modification, allows its functionalization at the surface level, which can confer different properties, among which may be antibacterial [19–21].

Taking into account some of the aforementioned aspects, cellulosic materials such as cotton and fique have been used to matrices to immobilize nanomaterials by different approaches [22–24], developing active packaging with biodegradable characteristics, which prevent or limit the proliferation of pathogens [4,16], where the antibacterial action of said containers has been attributed to the presence of impregnated nanomaterials on the cellulosic material, such as silver nanoparticles (AgNPs) [4,12,13,17,18]. Nanoparticles that can be obtained at different scales by physicochemical methods or by biosynthesis using fungi, these green chemistry methods are characterized by being environmentally friendly and more efficient from an energy point of view [25–27].

To date, the use of AgNPs obtained from fungi as an antibacterial principle for potential packaging for food storage and with the aim of contribute to the generation of new bio-based packaging with low environmental impact, this research employed AgNPs obtained from a native strain of *Fusarium oxysporum* after a bioreduction process of $AgNO_3$ a 3 mM, pH 10, 27 °C/24 h [28] that were immobilized on cotton fibers using two methodologies (cationization and *in situ* biochemical reduction) for further evaluation as antibacterial materials through quantitative techniques as a novelty. Then, using cationization as an anchoring methodology, a nanostructured material that is better adapted to actual potato storage conditions was developed; antibacterial fique sacks pilots. These sacks reduced the percentage of stored potatoes affected by the soft tuber rot disease compared to those that remained in traditional sacks during the trial. This novel application could contribute to managing the phytopathogenic agent *P. carotovorum* during large-scale potato storage, as it will significantly favor the product's safety and mitigate, to a great extent, the economic losses for producers, after performing some additional cytotoxicity tests to guarantee its safety.

2. Materials and methods

2.1. Obtaining AgNPs with antibacterial action from F. oxysporum

AgNPs were obtained from the enzymatic extract of a native strain of *F. oxysporum* that was selected after conducting a bioprospecting study in paramo soils cultivated with potato (*Solanum tuberosum*) in Boyacá-Colombia [28]. This strain, molecularly identified and cryopreserved on paper [29], showed the ability to produce AgNPs with uniform size distribution and with the best antibacterial activity against the phytopathogenic agent *P. carotovorum* concerning those obtained by other isolates.

Then, the methodology suggested by AbdelRahim et al. [30], with some modifications, was followed. The fungus was grown in potato dextrose broth (SDB) culture medium with agitation for seven days and at an incubation temperature of 28 °C. The biomass was harvested and washed with distilled water to prepare the mycelium-free cell filtrate (MFCF). For this, 10 g of biomass were mixed in 100 mL of Milli-Q water for 24 h under agitation at 28 °C to release the extracellular enzymes. After centrifugation at 5000 RCF/5 min to remove biomass, 100 mL of the supernatant was taken and mixed with 100 mL of a 3 mM $AgNO_3$ solution. The mixture was kept in the dark at 120 rpm/27 °C/24 h/pH 10 to facilitate nanoparticle synthesis. Then, after a color change of the mix and to verify the presence of silver nanoparticles in the solution, a UV-VIS spectrum was performed between 300 and 700 nm using distilled water as a blank to monitor the appearance of the maximum absorption band typical of this type of nanoparticles. Two experimental controls were used: the cellular filtrate free of mycelium (MFCF) and the $AgNO_3$ solution at 3 Mm.

2.2. Immobilization of AgNPs produced by F. oxysporum on cotton fiber

Cotton fibers were modified with AgNP using two methodologies; prior cationization of the fiber to obtain the "A-AgNPs-C″ material [31,32], and *in situ* biochemical reduction of silver to get the "A-AgNPs-IBR" material [33]. The first, A-AgNPs-C, were made by soaking 5 g of cotton fibers in 10 mL of Milli Q water, to later immerse them in a solution composed of 10 mL of 10 % NaOH and 5 mL of 75 % glycidyl trimethylammonium chloride (GTAC) solution for 15 min under agitation at 150 rpm. In this, GTAC binds quaternary ammonium groups under strongly alkaline conditions to the surface of the cotton fibers and thus obtains a cationized fiber. Subsequently, the fibers were rinsed with distilled water and air-dried, to finally take 5 g of cationized fiber, immerse them in Milli-Q water for 5 min, and immediately soak them in 10 mL of the AgNPs solution for 5 min more, and then rinse with Milli-Q water again. With which the assembly of the AgNPs obtained from *F. oxysporum* on the surface of these cationized fibers is obtained, due the electrostatic interaction between the fibers and negatively charged organic molecules found on the surface of the AgNPs obtained by biosynthesis [34].

Similarly, the A-AgNPs-IBR fibers were obtained by conditioning the cotton fiber using a solution of 8 % LiOH and 15 % urea under agitation at 150 rpm until a fibrous mass of cellulose was formed, to which absolute alcohol was added for 24 h. The fibers were then washed with distilled water to remove alcohol residues and thus obtain purified fibers for the *in situ* biochemical reduction process. Next, the fibrous matrix was placed in an Erlenmeyer containing 100 mL of $AgNO_3$ solution at a concentration of 3 mM for 12 h to favor

the absorption of silver ions into the fibrous structure. After this cotton-Ag^{+} formation, the fibers were washed with distilled water to remove silver ions not trapped in their structure. And then, the biochemical reduction was carried out *in situ*, placing 5 g of the cotton-Ag^{+} fibrous matrix in an Erlenmeyer containing 100 mL of the mycelium-free cell filtrate (FCLM) obtained from *F. oxysporum* as a reducing agent, thus getting the nanostructured fiber A-AgNPs-IBR.

2.3. Characterization analysis of cotton fibers modified with AgNPs produced by F. oxysporum

Scanning electron microscopy (SEM-EDS) characterized the two types of fibers to verify the presence of AgNPs and confirm the modification's efficiency. For this purpose, the fibers were fixed on a graphite tape, thinly coated with gold (Au) in the Denton Vacuum Desk IV equipment, and analyzed in the high vacuum scanning electron microscope to obtain high-resolution images. The secondary electron detector was used to evaluate the morphology and topography of the samples in the JEOL JSM 6490 LV equipment with an accelerating voltage of 10Kv. Additionally, XRD analysis was performed on the cotton fibers modified with AgNPs by the two methods (using an X-Ray Diffractometer BRUKER D8 Advance ECO). The equipment was configured in Bragg-Brentano geometry using a ceramic tube with copper (Cu) anode emitting a wavelength of 1.54 Å (Angstrom) with 40 kV and 25 mA for a power of 1000 W (Watts); the measurements were performed with a step magnification of 0.019° and a time/pass of 0.4s, the scanning was performed from 20° to 70° 2Theta (2θ). The codes of the crystalline phases detected in the samples were identified employing Profex software version 4.3.6 [35].

2.4. Antimicrobial activity of cotton fibers modified with AgNPs produced by F. oxysporum

The bacteria were grown in trypticase soy broth (TSB) under agitation at 28 °C/24 h until an OD of 0.1 was obtained, corresponding to 7.3 Log CFU/mL. The antibacterial effect of the fibers modified with AgNPs was evaluated by taking the inoculum and making five parallel streaks 6 mm wide and separated by 1 mm on nutrient agar Petri dishes. Then, 2 cm^2 fragments of the A-AgNPs-C and A-AgNPs-IBR fibers, previously sterilized, were taken and placed on the inoculated culture medium, applying pressure to ensure the contact of the fibers with the agar [36]. Finally, the Petri dishes were incubated at 28 °C/24 h, and the inhibition of bacterial growth was evaluated, using fragments of untreated sterile cotton as a control. Subsequently, a quantitative evaluation of the antimicrobial activity of the fibers was performed [37]. Fragments of 2 cm in length were taken from A-AgNPs-C and A-AgNPs-IBR fibers and placed separately in a vial containing 1 mL of sterile distilled water for 10 min. These vials were then spiked with 1.5 mL of nutrient broth and inoculated with a 10 μL aliquot of *P. carotovorum* bacterial suspension at 0.1 OD (7.3 Log CFU/mL). Then to evaluate the bactericidal activity of the cotton fibers, vials were incubated at 28 °C and 24 and 48 h, and plate counts were performed using a nutrient agar culture medium with incubation at 28 °C/48 h. The experiment's negative control was a vial without antimicrobial fibers, and all counts were performed in triplicate.

2.5. Reuse test of cotton fibers modified with AgNPs produced by F. oxysporum

The antimicrobial effectiveness of A-AgNPs-C and A-AgNPs-IBR fibers was evaluated during three consecutive uses by modifying the methodology suggested by Durán et al., [37]. Fragments of the two types of fibers, 2 cm long, were taken and placed separately in a vial containing 1 mL of sterile distilled water for 10 min. Subsequently, 1.5 mL of nutrient broth was added to these vials and inoculated with a 10 μL aliquot of *P. carotovorum* bacterial suspension at 0.1 OD (7.3 Log CFU/mL). The vials were incubated at 28 °C/48 h, using a nutrient agar culture medium to perform plate counts. Then, the antibacterial fibers already used were rinsed with sterile distilled water and re-evaluated under the same procedure two consecutive times. The experiment's negative control was a vial without antimicrobial fibers, and all counts were performed in triplicate.

2.6. Evaluation of the retention of AgNPs modified to cotton fibers produced by F. oxysporum

To determine the retention of AgNPs on the fibers, the method suggested by Durán et al. [37], was adjusted. For this purpose, 2 cm long fragments of A-AgNPs-C and A-AgNPs-IBR fibers were subjected to five successive washes with sterile distilled water. The effluent from these washes was collected to verify its antimicrobial action due to the presence of AgNPs released into the solution. 96-well microplates were used, and the wells under evaluation were inoculated with 50 μL of each effluent and 50 μL of the *P. carotovorum* bacterial culture at an OD of 0.1 (7.3 Log CFU/mL). The microplates were incubated in agitation at 28 °C/48 h, and their reading was performed at 590 nm after the incubation time. The absorbance value obtained in the calibration curve equation was then replaced by the number of Log CFU/mL to obtain the corresponding counts and verify the antibacterial action of the effluents evaluated, which is an indicator of the retention of the AgNPs on their supports. Inoculated culture medium was used as a positive control and sterile distilled water as a negative control. All readings were performed in triplicate. Finally, as an additional verification of the presence of AgNPs in the effluent product of each wash, a UV-VIS spectrum between 300 and 700 nm was performed on each sample to observe the maximum absorption of the AgNPs and the behavior of the absorbance.

2.7. Immobilization of AgNPs with antibacterial action on fique fibers as a pilot test of an antibacterial sack

Previous studies have shown that nanostructured materials such as fiber-based nanocomposites can be used under extreme temperature, humidity, and pressure conditions, without affecting their functionality [38]. Moreover, since potatoes in postharvest are

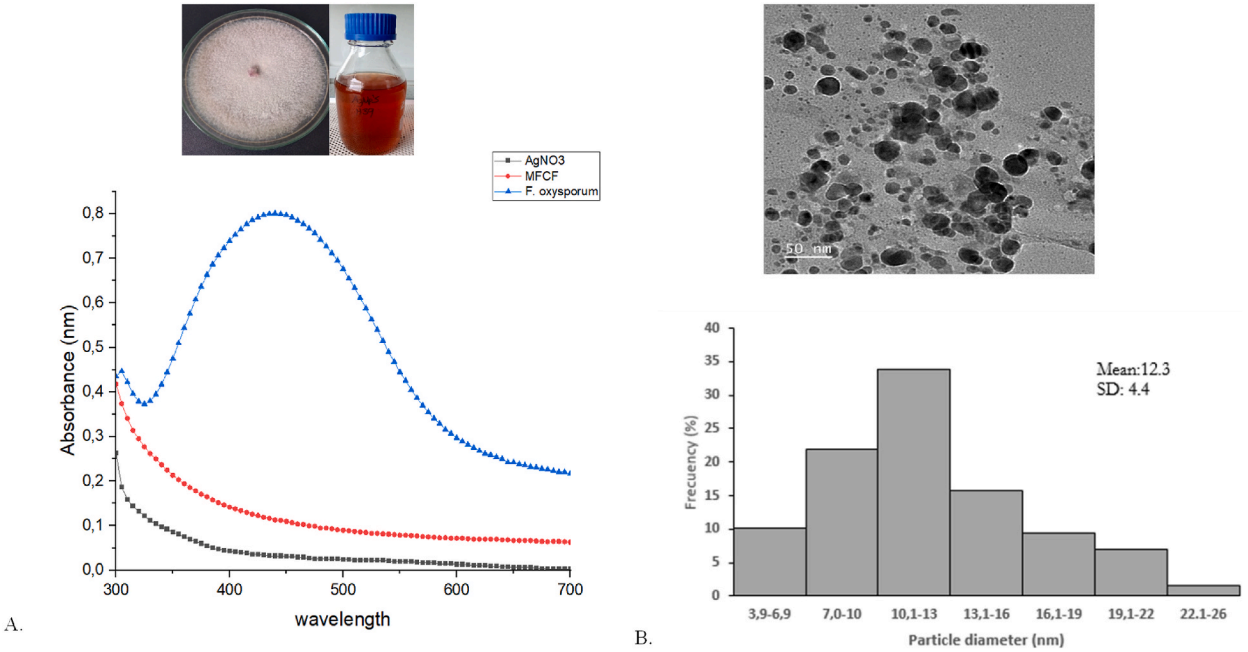

Fig. 1. A) Fungi, AgNPs and UV-VIS spectra, B) TEM micrograph and size distribution of AgNPs obtained from *F. oxysporum*. $AgNO_3$: 1 mM silver nitrate precursor solution. MFCF: mycelium-free cell filtrate.

generally stored in fique sacks, it was proposed to immobilize AgNPs with proven antibacterial action on this type of fiber to generate a packaging better adapted to actual conditions. For this, cationization was used as the immobilization methodology [31,32]. To verify the efficiency of the modification of fique fibers with AgNPs, characterization by scanning electron microscopy (SEM- EDX) and XRD analysis was performed. The cationization method was selected for its technical ease and for generating less leaching of the material, maintaining the antibacterial activity of the fiber. In addition, the structural heterogeneity of the porous surface of fique fibers is essential to facilitate the capture of AgNPs by this method [31].

2.8. Determination of the weight of macerated tissue from tubers stored in antibacterial sacks

After testing the efficiency of the Fique-AgNPs nanostructured material (*in vitro*), the potential antibacterial sack was fabricated following the cationization methodology but on a larger scale. Thus, Fique-AgNPs sacks with a dimension of 10 × 12 cm were developed, and with these, a final experiment with stored tubers (*in vivo*) was performed. This last trial was carried out to demonstrate the protective effect of the antibacterial sack on the development of the tuber's soft rot symptoms under more realistic potato post-harvest conditions. The trial included a positive control that had an infected potato stored in a traditional sterile sack (C+), a treatment that consisted of an infected potato in a sterile Fique-AgNPs antibacterial sack, and a negative control (C-) that was a healthy potato in a Fique-AgNPs antibacterial sack (Fique-AgNPs). The experiment was performed in triplicate. To perform the experiments, tubers of approximately 20 g were selected as experimental units, which were washed, disinfected with 70 % ethanol, and rinsed with sterile distilled water. Subsequently, infected tubers were immersed for 5 min in a culture of *P. carotovorum* at an OD of 0.3 (7.9 Log CFU/mL) as the infective dose. The treated tubers were placed in their respective packaging and kept at 28 °C/7 days. After the incubation, the weight of the macerated tissue in grams of each tuber was determined, and the percentage of affectation was calculated in each case. For the examination of results, an analysis of variance was performed to determine if there were significant differences between treatments and the Tukey test at 5 % for comparison between treatments.

2.9. Statistical analysis

An analysis of variance (ANOVA) and a 5 % Tukey test were performed to evaluate the antibacterial activity exerted by the fibers on the phytopathogenic agent, to verify their effectiveness after three consecutive uses, to evaluate the retention of AgNPs to their supports and to determine the percentage of affected tissue in tubers stored in the traditional and the antibacterial sacks.

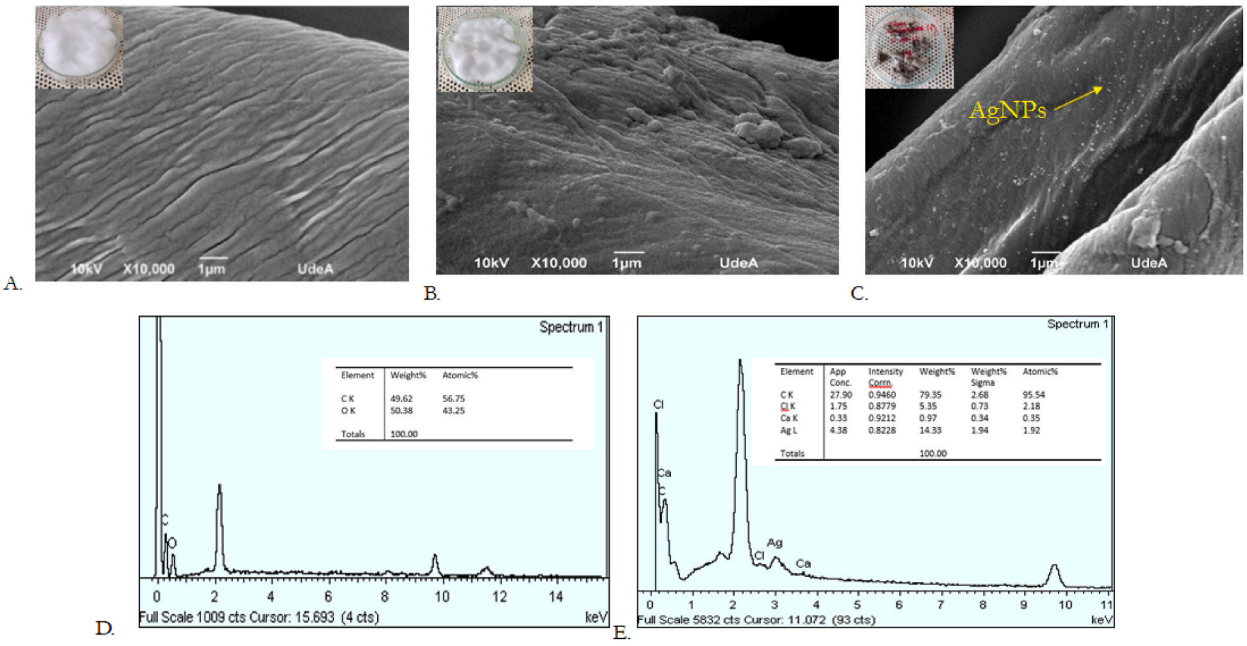

Fig. 2. SEM Micrographs of nanostructured fibers obtained by cationization as an anchoring methodology; A) Control (cotton fibers), B) Cationized cotton fibers, and C) Fibers A-AgNPs –C. EDX analysis of D) Control (cotton fibers); E) Fibers A-AgNPs –C.

3. Results and discussion

3.1. Obtaining AgNPs with antibacterial action from F. oxysporum

The UV–Vis spectrum of the obtained AgNPs indicated a single maximum absorption peak (Fig. 1A), indicating the production of isotropic nanoparticles with a narrow distribution [39]. These studies have suggested that the number of surface plasmon resonance (SPR) peaks increases as the nanoparticle symmetry decreases, therefore, for AgNPs of spherical shapes, an absorption maximum is expected, but for triangular nanoparticles, it is usual to find higher absorption máxima peaks [38]. The results of our experiments show an absorption maximum for AgNPs obtained from *F. oxysporum* located at 435 nm. Some investigations have reported that the maximum absorption peak of AgNPs synthesized by green chemistry appears at 380–420 nm [40,41]. However, in this case, the peak was located at a longer wavelength, which correlates with an increased size of the obtained AgNPs [42]. TEM analysis allowed evidencing spherical-shaped AgNPs with an average size of 12.3 ± 4.4 nm (Fig. 1B). These sizes are comparable to those reported by Husseini et al. [43], who using *F. oxysporum* obtained AgNPs between 5 and 13 nm, but are smaller than those reported for AgNPs obtained by Durán et al. [44], and Almeida et al. [45], who found nanoparticles in the range of 20–50 when working with the same fungal species. Other investigations employing other species of the genus *Fusarium* sp., like *F. culmorum*, *F. semisectum*, *F. scirpi*, and *F. solani* obtained spherical AgNPs in the range of 5–50 nm [46–50].

3.2. Immobilization of AgNPs produced by F. oxysporum on cotton fiber and their characterization

Several types of nanoparticles have been assembled on cellulose to impart catalytic or antibacterial activity [51]. Other studies have shown that cellulose fibers can be used as support during the synthesis of nanoparticles, employing *in situ* methods and the electrostatic assembly, making use of their nanoporous structure [22,23,52]. Therefore, the possibility of immobilizing AgNPs on these substrates is relevant as these nanocomplexes, or nanostructured materials, show superior antimicrobial activity than silver in a colloidal state [13,53]. Additionally, it has been reported that the potential toxicity of AgNPs can be prevented by being immobilized since their leaching rate to the environment is reduced, which generates benefits from an environmental and general health point of view [13].

3.2.1. Modification by cationization

After modifying cotton fibers with AgNPs employing cationization as an anchoring methodology, the fibers changed their coloration, turning brown. It has been reported that the impregnation of AgNPs to a substrate can be visualized by the yellow or brown coloration acquired by such substrates and that such coloration becomes darker with increasing AgNPs content [18,27,38,54–56]. To verify the efficiency of the modification of the A-AgNPs-C fibers, SEM micrographs were performed, and as observed in Fig. 2B, a change in fiber morphology occurred upon cationization concerning the control (Fig. 2A). This could be because the exposure of cellulose fibers to highly alkaline conditions can promote the destruction of intermolecular hydrogen bridges, with the consequent

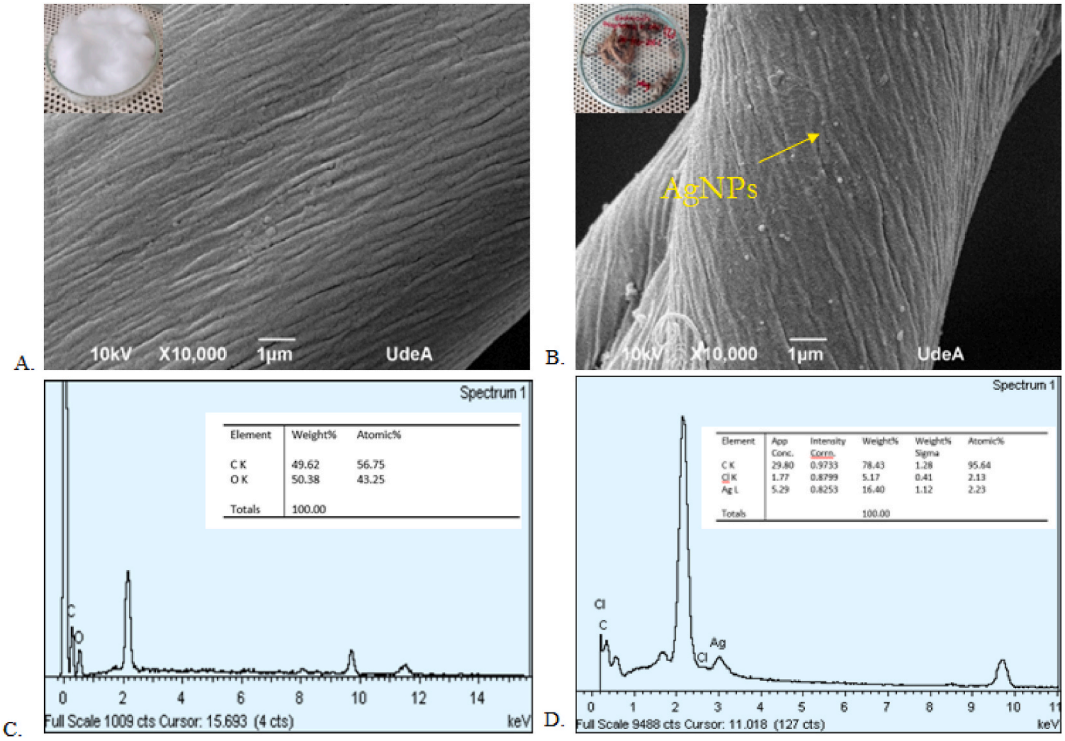

Fig. 3. SEM Micrographs of nanostructured fibers obtained by *in situ* biochemical reduction as an anchoring methodology; A) Control (cotton fibers), B) Fibers A-AgNPs- IBR. EDX analysis of C) Control (cotton fibers) and D) Fibers A-AgNPs– IBR.

affectation on their surface structure [55,57]. Additionally, in Fig. 2C, AgNPs are observed attached to the cellulose surface without the presence of aggregates, indicating successful modification. It has been reported that the cationization modification has the advantage that electrostatic interactions are established between the cationized cellulose and the negatively charged biosynthesized nanoparticles, which favors their retention [23,32].

The retention of AgNPs on a solid support is relevant since this favors their stability, preventing the AgNPs from forming aggregates, which easily occurs in AgNPs in colloidal suspension, thus losing their activity. In addition, the supports allow an appropriate release rate according to their subsequent application [58]. SEM-EDS analysis verified the elemental composition of the AgNPs present in fiber A -AgNPs-C, observing a silver signal at 3 KeV (Fig. 2D–E). The other elements found (Ca and Cl) are attributed to the industrial cotton bleaching process, where compounds such as calcium hypochlorite are used. This analysis was also performed by Emam et al. [38], who immobilized AgNPs obtained from xanthan gum on cotton fibers, finding the same characteristic signal at 3 KeV. On the other hand, Ahamed et al. [59], fabricated a biomaterial from the mixture of cellulose and chitosan impregnated with AgNPs with and without the antibiotic gentamicin as a dressing material for experimental wounds of rats. In that study, the EDS spectrum also showed the presence of silver with the signal corresponding to the 3 KeV.

3.2.2. Modification by in situ biochemical reduction

The *in situ* biochemical reduction involves the growth or formation of AgNPs directly on the fibers since the silver ions when mobilized inside the fibrous matrix, allow initiating the formation and growth process of the AgNPs. In the end, these are immersed in the fiber and on its surface; for this reason, it is considered an *in situ* reaction [23,60]. The naked eye could corroborate the formation of AgNPs in the cellulose matrix by the color change of the fibers that turned from gray to dark brown [33]. SEM micrographs (Fig. 3) allow verification that *in situ* biochemical reduction occurred on cotton fibers by using mycelium-free cell filtrate (MFCF) obtained from *F. oxysporum* as the reducing agent. Cellulose fibers have heterogeneous surfaces with many pores and cracks that can be used as nucleation and stabilization centers to synthesize AgNPs *in situ* [22,61]. The porous structure of cellulose stabilizes AgNPs thanks to the high density of oxygen surrounding the nanoparticle; this makes it possible to generate an effect similar to the so-called "crown ether effect", where oxygen atoms are firmly bound, forming ring-shaped chemical complexes with hydrophobic surfaces, where the cation is located inside the ring [62]. On the other hand, cellulose also has a reducing character thanks to the presence of OH groups, which could also favor the reduction process *in situ* [55,63].

On the surface of the fiber A-AgNPs-IBR, the AgNPs can be observed dispersed without aggregates concerning the control (Fig. 3A–B). The absence of AgNP aggregates on the fiber is ideal since it has already been reported that when AgNPs are clustered forming structures that exceed the nanometric range, they can lose their antibacterial activity, because their access to the pathogen would be reduced [13]. In this case, the negative z-potential of *F. oxysporum* AgNPs could have contributed to their repulsion, causing

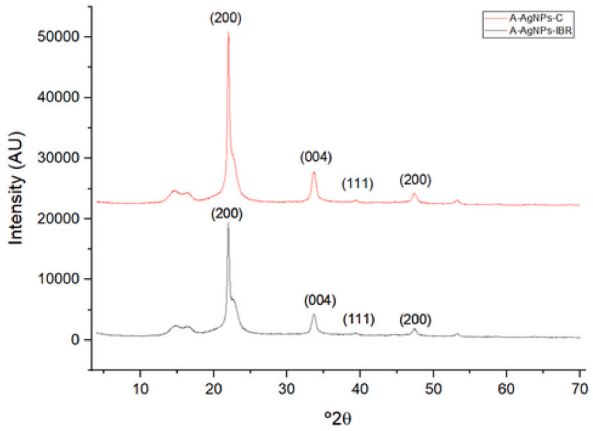

Fig. 4. X-ray diffraction analysis of AgNPs modified cotton fibers obtained from *F. oxysporum*.

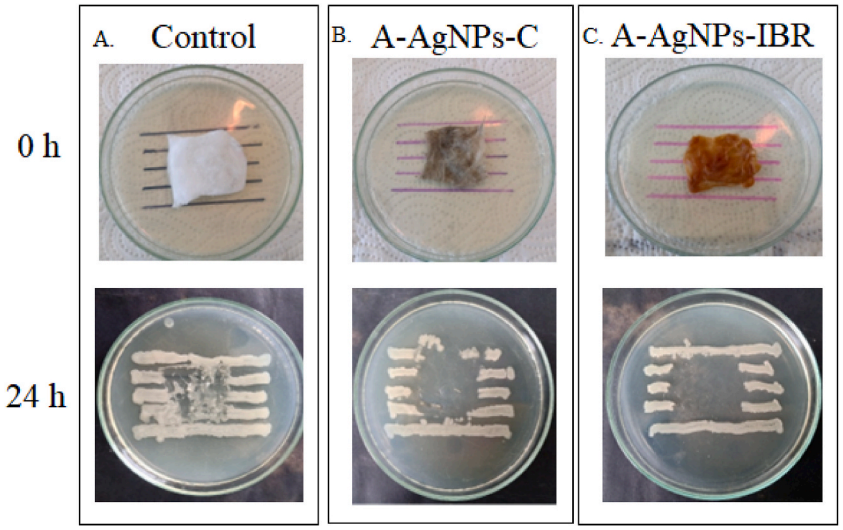

Fig. 5. Results of the agar diffusion test by the parallel streak method to verify the antibacterial activity of: A) Control cotton fibers, B) A- AgNPS–C fibers and C) A-AgNPs-IBR fibers.

them to remain dispersed even on the substrate.

These results differ from those reported by De Santamaría et al. [52], where SEM analysis indicates that AgNPs appear as aggregates of size greater than 100 nm with no defined morphology on the fibers. Likewise, Ibrahim et al. [64], observed clusters or groupings of AgNPs on the analyzed fibers due to aggregation processes despite having used ultrasonic processing before finishing, which favors the disintegration of AgNPs. Xu et al. [59], indicated that in their study AgNPs were not only embedded inside the fiber but also on the surface forming aggregates, which may have an impact on the antibacterial activity of the nanocomplex. SEM-EDS analysis verified the elemental composition of the AgNPs immobilized in the A-AgNPs-IBR fibers, observing a characteristic signal for the silver atom at 3 KeV regarding control (Fig. 3C-D), also observed for the AgNPs in suspension and for the A-AgNPs-C fibers.

On the other hand, the XRD analysis of the A-AgNPs-C and A-AgNP- IBR fibers allowed verifying the crystalline composition of the silver adhered to the cellulose surface. In both cases, similar diffraction patterns could be evidenced, with signals at angles 21.09° (200), and 33.7° (004) typical of cellulose, the main constituent of cotton fiber [13,65,66] and other diffraction angles at positions 39.4° (111) and 47.4° (200) corresponding to silver in a face-centered cubic crystal structure when immobilized on the plant substrate (Fig. 4). These same signals were reported by Ovalle et al. [61], when performing *in situ* synthesis of AgNPs on fique fibers and also by Arif et al. [63], who fabricated a cotton fabric with chitosan and silver nanoparticles whose XRD analysis showed the signals mentioned above for silver.

3.3. Antimicrobial activity of cotton fibers modified with AgNPs produced by F. oxysporum

The parallel streak method is a qualitative test that allows knowing the antibacterial potential of textile materials by placing them

Table 1
Quantitative evaluation of the antimicrobial activity of A-AgNPs-C and A-AgNPs-IBR fibers on *P. carotovorum*.

Fiber	Initial CFU/mL	Log CFU/mL counts at 24 h	Log CFU/mL counts at 48 h
A- AgNPs –C	7.3 ± 0.02^{a}	3.0 ± 0.01^{c}	$<1^{b}$
A-AgNPs-IBR	7.3 ± 0.02^{a}	3.1 ± 0.01^{b}	$<1^{b}$
control	7.3 ± 0.02^{a}	7.4 ± 0.001^{a}	7.4 ± 0.001^{a}

Reported counts are the average of three replicates ±standard error. At each evaluation time, equal letters do not differ significantly according to Tukey's 5 % test. < 1 Log CFU/mL is the limit of quantification of the technique [68].

Table 2
Reuse test results of A-AgNPs-C and A-AgNPs-IBR fibers.

Fiber	Log CFU/mL counts at 48 h		
	First use	Second use	Third use
A-AgNPs –C	$<1^{b}$	$<1^{b}$	1.1 ± 0.5^{b}
A-AgNPs- IBR	$<1^{b}$	$<1^{b}$	$<1^{b}$
control	7.4 ± 0.02^{a}	7.4 ± 0.02^{a}	7.4 ± 0.02^{a}

Reported counts are average of three replicates ±standard error. For each use, equal letters do not differ significantly according to Tukey's 5 % test. < 1 Log CFU/mL is the limit of quantification of the technique [68].

in contact with a culture medium inoculated with a bacterium to observe the growth inhibition resulting from its activity [36]. In this experiment, when removing the fragments of the different fibers under evaluation that were located on the microbial cultures, it was observed that the two types of fibers showed antibacterial activity since, in the areas of contact with the culture medium, bacterial growth was not observed, unlike what happened with the control fiber where bacterial growth was not inhibited (Fig. 5). Other researchers have determined the activity of nanocomplexes obtained by different routes against bacteria of clinical importance. Such is the case of Arif et al. [66], who evaluated the antibacterial activity of a material based on cotton, chitosan, and AgNPs obtained by traditional route using the parallel streak technique against Gram-negative bacteria such as *E. coli* and *P. aeruginosa* and Gram-positive bacteria such as *B. cereus* and *S. aureus*, also finding growth inhibition zones. Diez et al. [51], evaluated the antibacterial activity against *E. coli* of cellulose-fluorescent silver nanocomplexes, obtained traditionally, finding zones of inhibition that were produced by the release of silver ions or nanoclusters, indicating that several factors can affect the dissolution of silver ions to the medium, such as exposure time, temperature and pH of the agar.

Subsequently, a quantitative evaluation of the fibers' antimicrobial activity was performed to verify the antibacterial activity evidenced in the parallel streak test. In this experiment, viable cell counts were estimated after contacting the fibers (A-AgNPs-C and A-AgNPs-IBR) with a liquid culture of *P. carotovorum* for an incubation time of 24 and 48 h. Upon such counts, it was found that both types of fibers showed antibacterial activity since, at 24 h, there was evidence of a decrease in *P. carotovorum* counts, and at 48 h, there was complete inhibition of bacterial growth in all cases (Table 1). These results indicate the effectiveness of the two immobilization methodologies in obtaining nanocomplexes with antibacterial action, proving a synergy between the support and the AgNPs. The antibacterial action of AgNPs has been attributed to the release of silver ions as a result of an oxidative dissolution of the NPs once anchored to the fibers [67].

Most of the studies that evaluate the antibacterial activity of nanocomplexes use the bacterial reduction percentage as an indicator of said activity. Such is the case of Arif et al. [65], who determined this reduction of a material based on cotton, chitosan, and AgNPs, showing a decrease of 99 % against *S. aureus* and 98 % against *E. coli*. On the other hand, Ilic et al. [69], evaluated the antimicrobial efficiency of AgNPs obtained chemically against *E. coli*, *S. aureus*, and the fungus *C. albicans*, finding reduction percentages of 99.7, 99.9, and 99.8 %, respectively. Adepu et al. [13], prepared nanocomplexes of bacterial cellulose and silver nanoparticles by *in situ* reduction employing sodium borohydride ($NaBH_4$) as a reducing agent, these complexes showed 99.9 % antimicrobial activity against fungi and bacteria obtained from spoiled food, which was attributed to the prolonged release of AgNPs from the matrix. However, in our study, it was decided to perform for the first time the determination of bacterial reduction in terms of Log CFU/mL as an indicator of the antimicrobial activity of the fibers since it has been estimated that a material or substance that reduces bacterial counts by at least three log units can be considered a relevant antibacterial agent [70]. Based on the results, A-AgNPs-C and A-AgNPs-IBR fibers are efficient antibacterial materials. Notably, the use of this type of material for the control of a phytopathogenic agent has not been reported, results that are promising when it comes to generating antibacterial packaging for potato storage.

3.4. Reuse test of cotton fibers modified with AgNPs produced by F. oxysporum

It is essential to know the antibacterial effectiveness of the nanocomplexes after several uses as this is related to the useful life of the same. The results indicate that the cotton fibers modified by the two methods effectively retain AgNPs after three consecutive uses. However, for the A-AgNPs-C fibers, a slight decrease in their effectiveness can be observed in the third use (Table 2), this could be explained by the decrease in the load of NPs included in the fiber at the beginning of the experiment or because there was not enough leaching to continue generating the same antibacterial effect. Possibly, more reuse cycles are possible with our fibers. In that case, the antibacterial activity of the fibers will decrease, which follows what is suggested by Mohmed et al. [71], who indicate that the

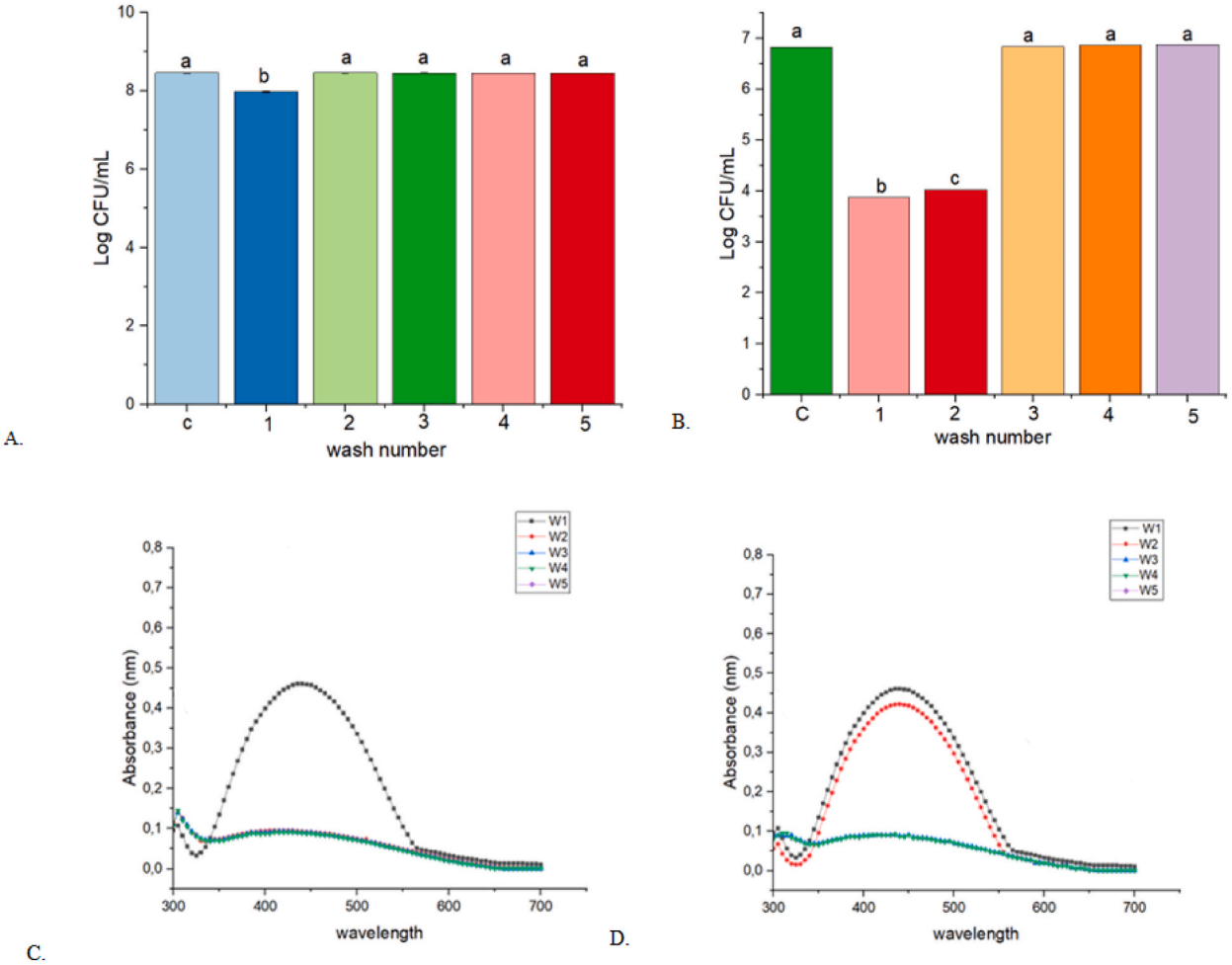

Fig. 6. Antibacterial activity of effluent from five successive fiber washes (W1–W5) as an indicator of AgNPs retention; A) A-AgNPs-C, B) A-AgNPs-IBR and UV-VIS spectra for AgNPs present in fiber wash effluent; C) A-AgNPs-C, and D) A-AgNPs-IBR after five successive washes (W1–W5). Bacterial counts are average of three replicates. Bars correspond to the mean average with their respective standard error. Equal letters do not differ significantly according to Tukey's test at 5 %.

antibacterial activity of antimicrobial fabrics is indirectly correlated with the number of washes.

To avoid loss of fiber activity after successive uses, Ilic et al. [69], suggest that, to achieve a long-lasting antimicrobial activity, fibers should be loaded with a large amount of AgNPs, as a higher concentration colloid provides an overall increase in the active silver surface area due to the high surface area/volume ratio of each nanoparticle. The excellent antimicrobial activity of A-AgNPs-C and A-AgNPs-IBR fibers after several uses demonstrate that there is a sufficient amount of Ag + ions or AgNPs released in a controlled manner to exert their antibacterial action over time [55]. These results raise the possibility that if potato storage packaging is made from these antibacterial fibers, it could be reusable, which also has positive environmental implications for the final disposal of such packaging.

3.5. *Evaluation of the retention of AgNPs modified to cotton fibers produced by F. oxysporum*

One of the main technical obstacles to modifying textile surfaces with AgNPs is their permanence, which is relatively low, especially against washing [55]. Our study observed that the two evaluated fibers showed differences in their retention rate of the immobilized AgNPs. For this experiment, it is essential to highlight that the presence of AgNPs in the effluent product of the washes is responsible for the antibacterial action against the phytopathogen. In this sense, for the A-AgNPs-C fibers, it can be observed that during the first washing, there was leaching of the immobilized AgNPs. Therefore, the bacterial counts are lower than those of the positive control of the experiment (untreated *P. carotovorum* culture). At the same time, for the A-AgNPs-IBR fibers, leaching of the nanomaterial was evidenced during the first two washes. That is why only until the third wash was the bacterial counts equal to those of the positive control (Fig. 6A–B). On the other hand, UV–vis spectra were performed on the effluents to verify the previous results, which allowed monitoring of the AgNPs in the solution. It was found that the A-AgNPs-C fibers released a lower amount of AgNPs into the effluent

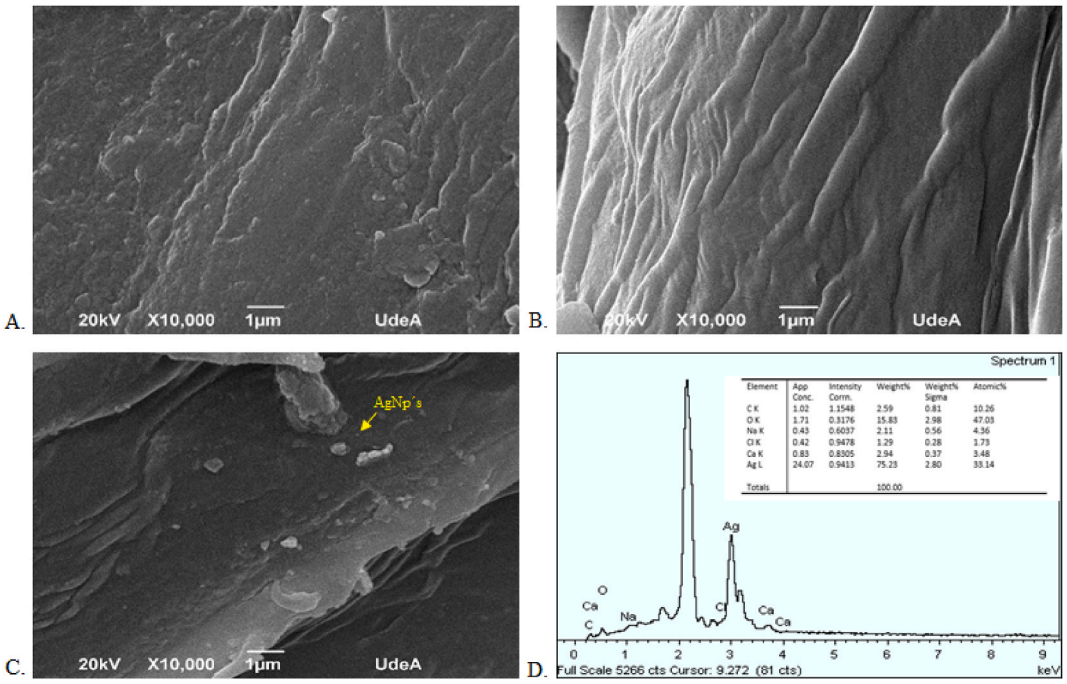

Fig. 7. SEM micrographs of; A) Untreated control fique fibers, B) Cationized fique fibers, C) Fique-AgNPs fibers, and D) SEM-EDX analysis of the Fique-AgNPs fibers.

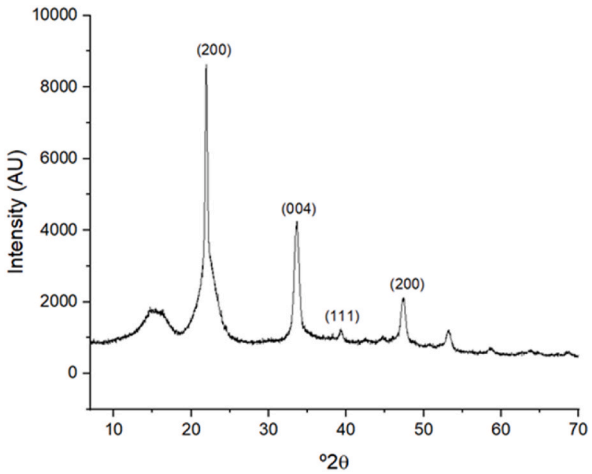

Fig. 8. X-ray diffraction analysis of fique-AgNPs fibers.

since only until the second wash was there a decrease in absorbance units at the maximum absorption associated with the presence of AgNPs. While for the A-AgNPs-IBR fibers, more significant leaching of AgNPs to the effluent product of the washings was evidenced (Fig. 6C–D), all of the above would explain the antibacterial action that the effluents evaluated during the test showed.

These results are related to the antibacterial efficiency of the fibers after several washing cycles. In that case, the most efficient material would be the A-AgNPs-C fibers. This parameter has been evaluated in different types of nanocomplexes by some authors finding that even after 20 washes, the fibers still exert an antibacterial action of at least 50 % concerning that presented before the washes [38,65]. In this regard, it has been indicated that such efficiency could be increased if a binding agent or binder is added to the treated fibers in the process, such as acrylate [72], or if high temperatures are used during the coating process of the fibers with the AgNPs as this increases their adhesion to the support [55]. Finally, although the concentration of leached AgNPs was not quantified in this study, this retention test of AgNPs to the fibers could suggest that the leaching rate was low. This is relevant if a potato storage package is manufactured due to the potential toxicity of AgNPs and their health implications. In this sense, the study conducted by Avella et al. [73], showed that the migration of AgNPs from a starch-clay nanocomplex used to store vegetables was minimal. However,

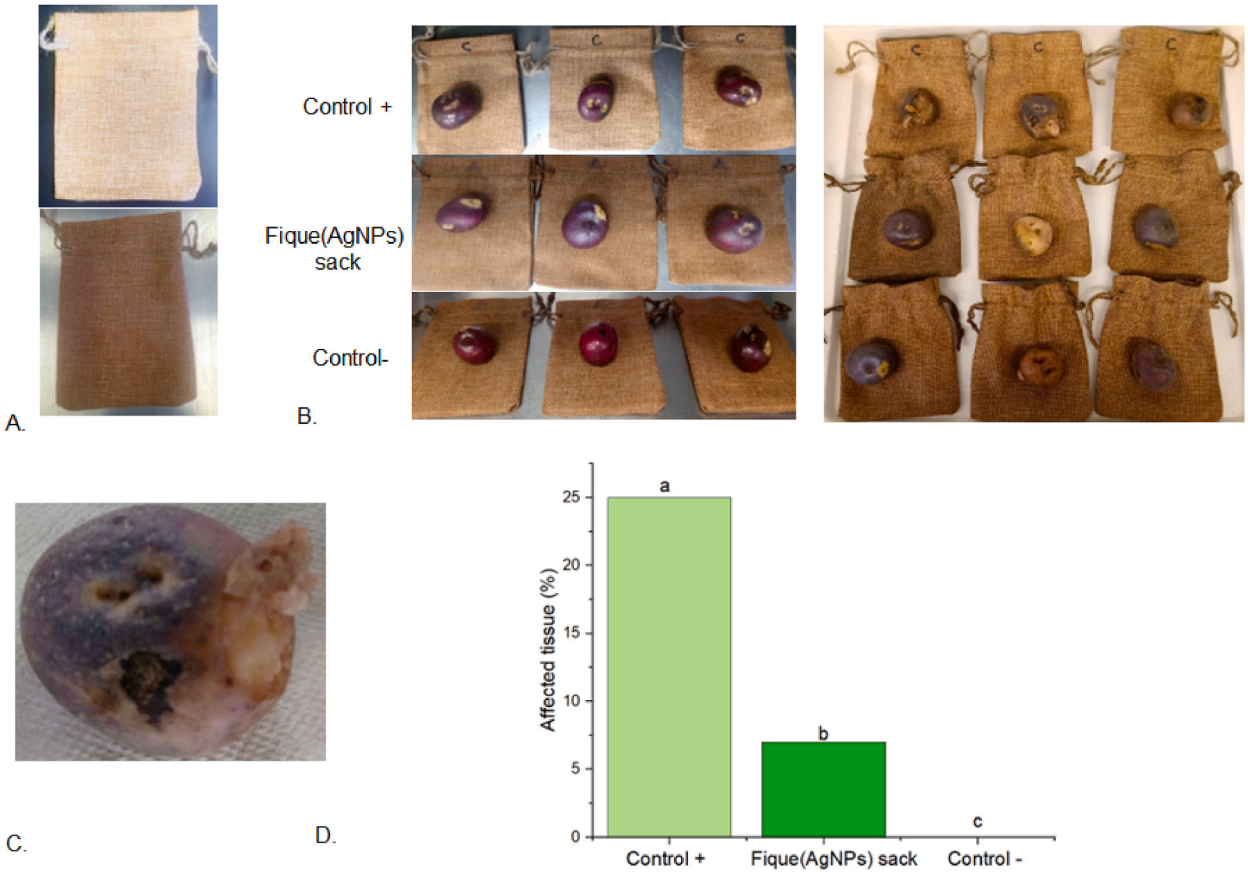

Fig. 9. Photographs of; A) Traditional sack (top) and Fique-AgNPs antibacterial sacks (bottom), B) Set-up and the results of the experiments for determination of macerated tissue weight of *P. carotovorum* infected tubers stored in antibacterial and traditional sacks C) Tissue maceration in *P. carotovorum* infected tuber, and D) Percentage of affected tissue of tubers stored in traditional sacks (C+) and in Fique-AgNPs antibacterial sacks. The percentage of affected tissue of tubers is the average of three replicates. The bars correspond to the average mean with its respective standard error. Equal letters do not differ significantly according to Tukey's test at 5 %.

the study clarifies that more research is needed to reach a conclusive statement.

3.6. Immobilization of AgNPs with antibacterial action on fique fibers as a pilot test for an antibacterial sack

When performing the immobilization of AgNPs on fique fibers to develop an antibacterial sack as a potential application for potato storage, it was observed that the fique fibers changed coloration to a dark brown color, suggesting that the AgNPs have been immobilized [32]. However, to verify the success of the process, SEM analysis was performed on the fique fibers in each of the stages of the method, which are the cationization and subsequent anchoring of AgNPs. Initially, after the cationization process, a change in the morphology of the fibers was observed since they lost their roughness, and their surface became more uniform concerning the control (Fig. 7A–B). Then, during the AgNPs assembly process, some bright spots were observed on the fique fibers, which are associated with the presence of AgNPs (Fig. 7C). Finally, the presence of silver on the surface of the fique-AgNPs could be verified by SEM-EDS analysis (Fig. 7D).

Similarly, the X-ray diffraction analysis of the fique-AgNPs fibers proved the crystalline nature of the immobilized AgNPs, presenting the same diffraction pattern as reported for the cotton nanocomplexes (Fig. 8). Ovalle et al. [61], synthesized a nanocomplex material by *in situ* deposition of AgNPs on fique fibers, confirming by XRD analysis the presence of silver on the substrate, since in the diffractogram they observed signals corresponding to silver, with a cubic crystalline structure centered on the faces at angles 38.08° (111) and 47.30° (200).

3.7. Determination of the weight of macerated tuber tissue stored in antibacterial sacks

A final potato storage trial was conducted under controlled conditions to verify the antibacterial action of the fique-AgNPs antibacterial sacks pilots (Fig. 9B). As mentioned, soft rot symptoms on the tuber include plant tissue maceration (Fig. 9C). For that reason, this parameter was defined as the response variable of the experiment. It was found that after storage time in the two types of sacks

pilots (Fig. 9A), differences were found in the weight of macerated tissue and the percentage of affectation of each tuber ($p \leq 0.05$). For tubers stored in the traditional sack, an average macerated tissue weight of 5 g was found, corresponding to an affectation percentage of 25 %, and for tubers stored in the Fique-AgNPs antibacterial sacks, a macerated tissue weight of 1.6 g was found, corresponding to an affectation percentage of 7.8 % (Fig. 9D).

With these preliminary results of the effectiveness of the Fique-AgNPs antibacterial sacks, the basis is laid for more extensive scale studies. This development can contribute to the postharvest storage state of potatoes to protect the food not only against the phytopathogen *P. carotovorum* but also against other phytopathogens since AgNPs have been shown to have another type of pesticidal action.

4. Conclusions

In this research, AgNPs obtained by mycosynthesis from *Fusarium oxysporum* were immobilized on cotton fibers using two methodologies that allowed the preparation of fibers called A-AgNPs-C and A-AgNPs-IBR. These fibers were evaluated as antibacterial materials to control the phytopathogenic agent *Pectobacterium carotovorum* by inhibiting its growth. These fibers were found to be effective after three successive uses, regardless of the modification method employed. It was also verified that AgNPs remain attached to A-AgNPs-C and A-AgNPs-IBR fibers after two and three consecutive washes. By immobilizing AgNPs on fique fibers by cationization, it was possible to manufacture the pilot of an antibacterial packaging for potato storage that allowed reducing the percentage of macerated tissue from 25 % that resulted after storing the tubers in traditional sacks to 7.8 %. In addition to its antibacterial effectiveness, this sack would be biodegradable and have a low environmental impact. However, it is necessary to carry out some additional cytotoxicity tests to guarantee its safety.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Mayra Eleonora Beltrán Pineda: Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Luz Marina Lizarazo Forero:** Writing - review & editing, Supervision. **Cesar A. Sierra:** Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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