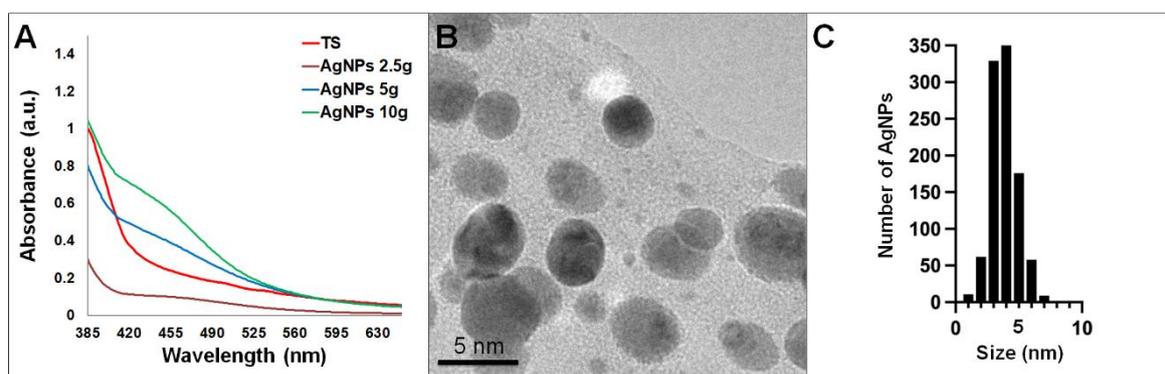


Results obtained and considered for optimized protocol

### 1. Biomass required for the synthesis of AgNPs using supernatants

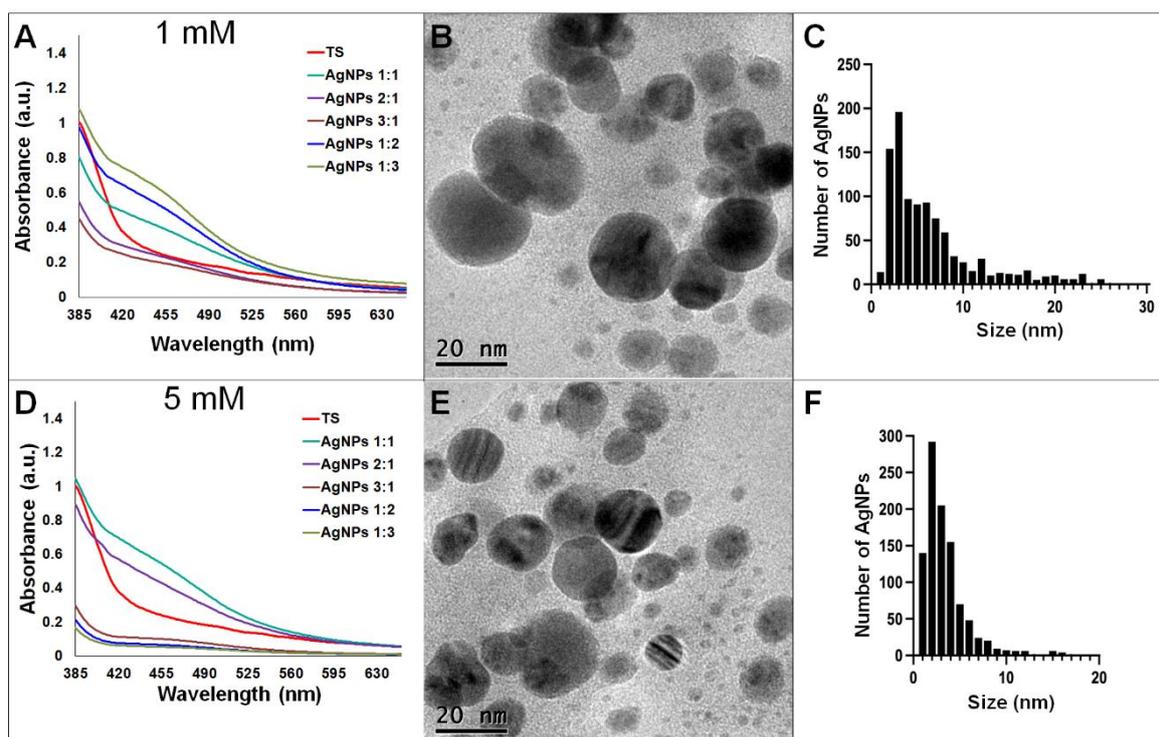
In order to optimize the synthesis, different amounts of *T. harzianum* biomass (2.5, 5 and 10 g) were used to obtain supernatants. The resulting supernatants were utilized to synthesize of AgNPs using a ratio of 1:1 (reducing agent/ 5mM AgNO<sub>3</sub>) and incubated at room temperature. After 24 h, the characteristic change of color from clear to light brown occurred in all reactions. By UV-Vis spectroscopy the characteristic curves of absorbance between 400-500 nm, associated with the typical Surface Plasmon Resonance (SPR) for silver nanoparticles were obtained, indicating successfully formation of AgNPs (Figure 1A). However, absorbance was higher using 10 g of biomass compared with 2.5 and 5 g, indicating a higher concentration of AgNPs. Therefore 10 g of fungal biomass, incubated in 100 mL of deionized water were used to obtain the supernatant for subsequent optimization protocol. After the incubation time, AgNPs obtained using 10 g of biomass were analyzed by TEM and NPs obtained were quasi-spherical in shape (Figure 1B) with an average size of 3.8±1.0 nm and range size of 1 to 7 nm (Figure 1C).



**Figure S1.** Synthesis of AgNPs after 24 h at room temperature using the supernatant of *T. harzianum*. (A) UV-Vis absorption curves obtained using the supernatants of different amounts of biomass and 5 mM AgNO<sub>3</sub>, (B) TEM micrograph of AgNPs obtained using the supernatant of 10 g of biomass, (C) corresponding size distribution histogram. TS = *Trichoderma* supernatant.

### 2. Synthesis of AgNPs using fungal supernatant at different concentrations of AgNO<sub>3</sub>

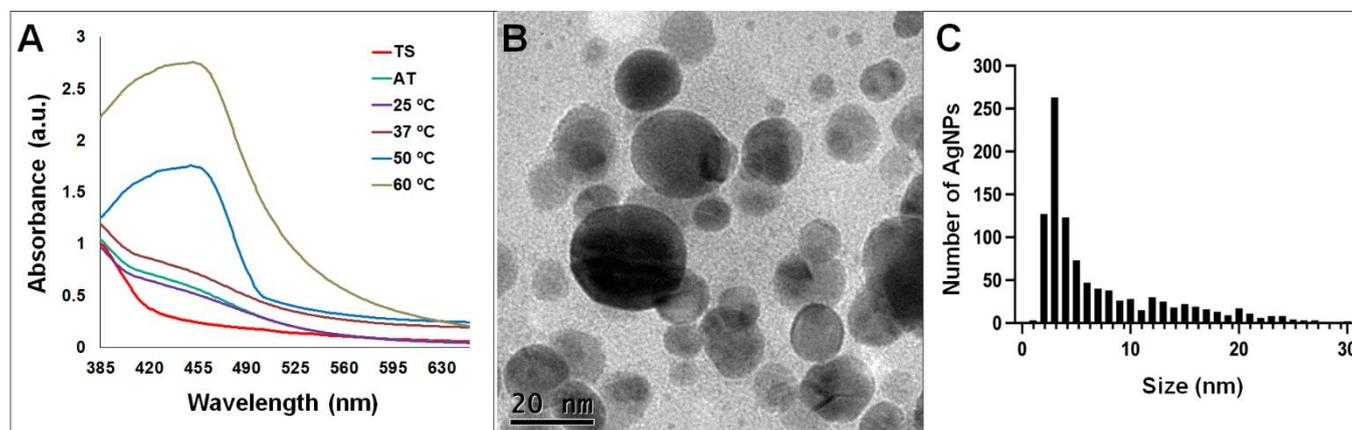
The synthesis of AgNPs using the supernatant of *T. harzianum* (from 10 g of biomass incubated in deionized water) was carried out at ambient temperature using different concentrations of AgNO<sub>3</sub> and different proportions of both the supernatant and the metallic precursor. The samples were first analyzed by UV-Vis spectroscopy and the absorption bands obtained are shown in Figure 2. We determined that using 1 mM of AgNO<sub>3</sub>, the formation of NPs was more evident using a 1:3 ratio (supernatant, AgNO<sub>3</sub>) (Figure 2A), while using a concentration of 5 mM, a similar curve of absorbance was obtained with a 1:1 ratio (Figure 2D). Analysis by TEM revealed that small quasi-spherical NPs were produced using both concentrations of AgNO<sub>3</sub> (Figure 2B, 2E); however, using 1 mM slightly larger NPs were obtained. Average size for obtained NPs using 1 mM of AgNO<sub>3</sub> and a ratio of 1:3 was 6.5 ± 5.1 nm in size, with a size range of 1 to 25 nm (Figure 2C). The synthesis of nanoparticles using a concentration of 5 mM and a ratio of 1:1, produce particles with an average size of 3.5 nm ± 2.7 nm in size, and a size range of 1 to 16 nm (Figure 2F). For subsequent syntheses, a ratio of 1:3 (reducing agent, 1 mM AgNO<sub>3</sub>) was used, since similar results in size and shape were obtained using 1 and 5 mM of AgNO<sub>3</sub>.



**Figure S2.** Synthesis of AgNPs at room temperature using the supernatant of *T. harzianum* and different proportions of supernatant:AgNO<sub>3</sub>. (A-C) Results obtained using 1mM AgNO<sub>3</sub>; (D-F) Results obtained using 5mM AgNO<sub>3</sub>. (A, D) UV-Vis absorption curves of AgNPs; (B, E) TEM micrographs of AgNPs obtained using proportions (1:3) and (1:1), respectively and (C, F) corresponding size distribution histograms. TS = *Trichoderma* supernatant.

### 3. Synthesis of AgNPs using fungal supernatant at different temperatures

The synthesis of AgNPs at ambient temperature was monitored every day by UV-Vis spectroscopy, and it was after seven days that no further change was observed (data not shown). Therefore, in order to accelerate the reaction, the supernatant of *T. harzianum* was used to carry out the synthesis at different temperatures, using a ratio of 1:3 and 1 mM AgNO<sub>3</sub>. Higher absorbance was detected when using 60°C (Figure 3A). Analysis by TEM of NPs obtained at this temperature revealed quasi-spherical NPs (Figure 3B), with an average size of  $7.1 \pm 5.9$  nm and a size range of 1–30 nm (Figure 3C). Therefore, subsequent syntheses were carried out at 60°C.



**Figure S3.** Synthesis of silver nanoparticles using the supernatant of *T. harzianum* at different temperatures. (A) UV-Vis absorption curves obtained at different temperatures, (B) TEM micrographs

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of AgNPs synthesized at 60°C, (C) corresponding size distribution histogram. TS = *Trichoderma* supernatant, AT = ambient temperature.