**Objectives**:Currently available drugs for the treatment of cryptococcosis are scarce, as well as expensive and usually associated with relevant side effects. Additionally, *Cryptococcus neoformans* not only has shown increasing resistance to classical antimycotics, but also is able to form biofilms on medical devices, posing significant challenges to pharmacological treatment. Thus, ongoing research aims to develop new antifungal agents and to improve existing therapies. Silver nanoparticles (AgNPs) have been suggested as promising anticryptococcal agents, and biogenic AgNPs stand out for being synthetized in simple, fast, cost-effective, and eco-friendly manners. Particularly, fungal-mediated AgNPs synthesis (mycosynthesis) offers biotechnological benefits, such as colloidal stabilization of AgNPs through the formation of a biomolecular capping influenced by both the fungal species and the growth medium used. Herein, three different mycosynthesized AgNPs were produced and characterized, and further screened in vitro for antifungal and antibiofilm activities against *C. neoformans*.

**Materials & Methods:** Mycosynthesis of AgNPs was performed using the mycelia of *Phanerochaete chrysosporium*, *Penicillium expansum* and *Punctularia atropurpurascens* grown in Malt Extract Broth. Three different AgNPs were successfully obtained and were physiochemically characterized through a combination of several techniques; including: dynamic light scattering (DLS), Z-potential determination, nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and capping-protein quantification. Biocompatibility profiles of AgNPs were further assessed *in vitro* through determination of intrinsic hemolytic and cytotoxic activities (HA50 and CA50, respectively). Then, the antifungal activity of AgNPs was screened *in vitro* using the H99 strain of *C. neoformans* through determination of both the minimal inhibitory concentration and minimal fungicidal concentration (MIC and MFC, respectively; broth dilution technique and CFU counting). Finally, the antibiofilm potential of AgNPs was assessed against H99 cells using the crystal violet assay for the study of biofilm inhibition (minimal biofilm inhibitory concentration, MBIC) and/or biofilm eradication activity.

**Results**: The three mycosynthesized AgNPs were spherical (TEM), with sizes ranging from 14 to 78 nm (DLS), Z-potential values between -18.5 and -22.9 mV, and capping-protein content in the range 4-45 fg/AgNP. Additionally, the three AgNPs showed good biocompatibility profiles, exhibiting HA50 values in the range 6.5x107-5.9x108 AgNPs/mL, and CA50 values from 3.0x108 to >1.0x109 AgNPs/mL. Then, regarding anticryptococcal activities, two AgNPs showed fungicidal effects against H99 cells: those derived from the mycosynthesis by *P. expansum* (MFC = 5.0x108 AgNPs/mL) and by *P. chrysosporium* (MFC = 2.0x109 AgNPs/mL). Finally, although the three AgNPs showed biofilm eradication activity when assayed at 1x1010 AgNPs/mL, only the two fungicidal AgNPs displayed also biofilm inhibitory activity (MBIC values of 6.2x107 and 2.9x107 AgNPs/mL for *P. expansum* and *P. chrysosporium* derived AgNPs, respectively).

**Conclusions**: The obtained AgNPs displayed physicochemical and biological differences depending on the fungal species used for the mycosynthesis. Among the synthesized and assayed AgNPs, those obtained from *P. expansum* stood out because of showing good fungicidal and biofilm inhibition activities, while exhibiting the highest biocompatibility profiles. Therefore, mycosynthesis using *P. expansum* mycelium showed to be a suitable source for obtaining promising biogenic AgNPs to further evaluate as potential novel anticryptococcal agents.