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In vitro assessment of a nanoparticle-based fungicide against *Monosporascus cannonballus*

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Abstract

The melon (*Cucumis melo* L.) holds significant socioeconomic importance in Brazil, particularly in the Northeast. However, increasing production intensity has worsened phytosanitary issues, especially due to *Monosporascus cannonballus*, a major pathogen that causes root rot. Conventional control methods, mainly relying on cultural management and pesticide use, are often ineffective and environmentally harmful. This study introduces and characterizes a nanoparticle-based fungicide combining Arbolina® and fludioxonil to inhibit the causal agent of root rot. Nanoparticle characterization, both with and without fungicide, showed differences in particle size, surface charge, and UV-Vis absorption, indicating successful fungicide incorporation into the nanoparticle matrix. *In vitro* bioassays suggest that the nanoparticles primarily enhance fungicide delivery and stability, rather than providing direct antifungal action. This formulation effectively inhibits the fungal growth with concentrations of 100, 400, 800, 1200, and 1600 mg/L achieving inhibition rates between 98.93% and 100%. Our findings demonstrate that Arbolina® is an effective carrier for fungicides, enhancing their efficacy against *M. cannonballus in vitro*.

1. Introduction

The Brazilian Northeast stands out with a percentage of 98,03% in the production of melon (*Cucumis melo L.*), with the states of Rio Grande do Norte (RN) and Ceará (CE) responsible for 77,74% of this production (IBGE, 2024). In this region, the production is linked to the use of agricultural inputs, especially chemicals, and the cultural practices adopted have been responsible for causing phytosanitary problems (Bruton et al. 1998; Sales Jr. et al. 2018), which are the factors that inhibit the expansion of the crop. Among the main pathogens the fungi *Monosporascus cannonballus* which causes root diseases in melon plants, *Monosporascus* root rot and vine decline (MRRVD) in melon.

Monosporascus cannonballus Pollack & Uecker among other soil-borne pathogens are the causal agent of root rot, a serious threat to melon crop production. *M. cannonballus* appears adapted to hot, semiarid, and arid climates and grows optimally at higher temperatures over 25°C (Sales Jr. et al. 2018). Infected melons typically exhibit symptoms such as root discoloration, wilting, stunted growth, and vine decline, especially under high soil moisture and temperature conditions. The fungi invade the roots, disrupting water and nutrient uptake, which ultimately causes plant collapse. Effective management includes resistant cultivars, soil solarization, crop rotation, and biocontrol agents (ex.*Chaetomium*) to reduce pathogens and enhance plant resilience.

Recently, nanotechnology is revolutionizing agriculture, with carbon quantum dots (CQDs) emerging as a promising tool to enhance crop productivity and sustainability. Moreover, CQDs show their potential as biostimulants, improving plant growth under stress conditions (Vieira et al. 2023). Due to their remarkable structural versatility, CQDs have found applications across numerous research fields, including biomedicine, agriculture, and catalysis. Among CQDs products a Brazilian technology was developed and commercialized, Arbolina®, a naturally derived eco-friendly CQDs (Rodrigues et al. 2023).

Nanoparticles can act as highly efficient carriers for natural and synthetic active molecules, presenting a promising innovation for integrated disease management strategies (Shah & Wani, 2016; Shahzad et al. 2024). These versatile carriers enhance the delivery and bioefficacy of active ingredients, REFERENCE. Within this framework, we investigated the use of carbon quantum dots (CQDs) as carriers for fludioxonil, a widely utilized fungicide, and evaluated their performance in vitro against *M. cannonballus*. The study aimed to develop an alternative approach by integrating nanoparticles with commercial formulations, potentially enhancing their efficacy while mitigating environmental impact.

2. Materials and Methods

2.1. Biological Material and chemicals

The CQDs Arbolina® was provided by Krilltech (www.krilltech.com.br) as a product prototype in the solid state. The active ingredient is a non-systemic fungicide and protector with depth action, Fludioxonil [-4-(2,2-difluoro-1,3-benzodioxol-4-yl) -1H-pyrrole-3-carbonitrile] at a concentration of 25 g/L.

The *Monosporascus cannonballus* isolate was collected from melon crop roots in Rio Grande do Norte State, Brazil, and identified using molecular techniques (Negreiros et al. 2019). It is stored at UFERSA's Phytopathology II Laboratory and the “Prof. Maria Menezes” Collection (CMM 2429). For bioassays, the isolate was cultured on potato dextrose agar (PDA, Merck) at 28°C in the dark for 7–10 days.

2.2. Particles Characterization

Arbolina® and its combination with fludioxonil were characterized using Fourier-transform infrared spectroscopy (FTIR), Zeta Potential (ZP), electron microscopy, and particle size analysis. For FTIR, 2 mg of the sample was mixed with 198 mg of potassium bromide (KBr), pressed into a pellet, and analyzed with a Spectrum Two FTIR spectrometer (PerkinElmer, USA) over 4000–400 cm^{-1}. Particle size and ZP

measurements were performed with a Zetasizer Nano ZS90 (Malvern Instruments, UK) at 25 °C after diluting the samples (1:100 or 1:1000 v/v) and stabilizing for 120 seconds. UV-vis spectra were obtained using a Varian Cary 50 UV-Vis spectrophotometer (Agilent Technologies, USA), measuring absorbance from 200 to 800 nm.

2.2. 1 High-Resolution Transmission Electron Microscopy (HRTEM).

HRTEM imaging was performed using a ZEISS high-resolution microscope. A drop of 20 mg/L Arbolina® suspension was placed on a TEM grid, treated with phosphotungstic acid for contrast, and examined at 80 kV with magnifications above 5000x to observe nanoparticle morphology.

2.3. Bioassays

The *in vitro* bioassays were conducted at the Phytopathology Laboratory at Embrapa Agroindústria Tropical, Fortaleza, Ceará. To evaluate the inhibition of mycelial growth (IMG) of *M. cannonballus*, mycelial growth was measured after seven days of incubation at 28°C in darkness using a digital caliper, and IMG (%) was calculated using the formula:

$$IMG\% = 100 - \left(\frac{D_c - D_t}{D_c}\right) \times 100$$

, in which: Dc = control diameter; Dt = treatment diameter.

2.4. Fludioxonil Dose-Response

Fludioxonil's impact on mycelial growth was assessed using a completely randomized design (CRD) with five treatments (0, 0.1, 1, 10, and 50 mg/L) and five replications, totaling 25 plots. PDA medium with fungicide was autoclaved, poured into Petri dishes, inoculated with *M. cannonballus*, and incubated for seven days.

2.5. Combined Nanoparticle and Fludioxonil Treatments

Twelve treatments combining Arbolina® (NP) and fludioxonil (FX) at varying concentrations (e.g., 1.620 mg/L NP + 0.1 mg/L FX) were tested in CRD with five replications, totaling 60 plots. Treatments included nanoparticles alone, fungicide alone, and a control. The mixtures were incorporated into PDA, inoculated with *M. cannonballus*, and incubated for seven days to measure mycelial growth under controlled conditions.

3. Results

Molecular Characterization

Arbolina® was characterized with and without the fungicide revealing differences in particle size, surface charge, and UV-vis absorption, and also indicating fungicide incorporation and interaction with the nanoparticle matrix (Figure1). The FTIR spectrum showed absorption bands at 1700, 1650, 1400, and 1180 cm−1, linked to C=O, C=C, C-N, and C-O bonds, with additional bands at ~2900 and 3400 cm−1 for C-H and O-H/N-H bonds. ZP analysis revealed a nanoparticle size of 15 nm and a negative ZP of −18.0 mV, due to -OH and -COOH groups on its surface (Figure 1A).

The particle size of the formulated nanoparticle (182.5 nm) was compared with that of fludioxonil alone. The results, shown in Figure 1B, indicated no significant increase in particle size. This finding suggests that the nanoparticle was successfully incorporated into the formulation without altering the fungicide's size distribution or compromising its molecular structure. To further evaluate the formulation's stability, Zeta Potential (ZP) analysis was performed after combining the nanoparticle with fludioxonil (Fig. 1C). The ZP increased to -37.8 mV, reflecting an improvement in stability (Lowry et al. 2016). This higher ZP value indicates enhanced electrostatic repulsion between particles, reducing the likelihood of aggregation and promoting better dispersion of the nanoparticles within the fungicide matrix.

The UV-vis absorption spectra of the nanoparticle-fludioxonil formulation revealed a significant increase in absorption compared to the spectra of the individual components (Fig1D). This result suggests a strong interaction between the nanoparticle and the fungicide, likely due to fludioxonil adsorbing onto the nanoparticle's surface. High-resolution transmission electron microscopy (HRTEM) images captured at 85,000X magnification revealed a mixture of nanoparticles and aggregated particles with elongated and heterogeneous morphologies. The sizes of the functional nanoparticles ranged from 10 to 50 nm. (Figure 1E).

Bioassays

Assessment of In Vitro Mycelial Growth Suppression in Monosporascus cannonballus: Fludioxonil Dose-Response Standardization

The linear regression analysis indicated no statistically significant effect of experimental repetitions. However, a significant relationship was identified between the treatments and the inhibition of mycelial growth (IMG) by in vitro bioassays with *Monosporascus cannonballus*. The regression model for IMG included experimental repetition as a factor to evaluate the dose-response standardization of Fludioxonil in controlling *M. cannonballus* (Figure 2a). The treatments using the IC50 of fludioxonil effectively inhibited fungal growth, achieving 100% Mycelial Growth Inhibition (MGI) for all cases showed a significant predictive effect, with a coefficient of determination (R^2)(Figure 2b). On the other hand, the treatments with Arbolina® at several concentrations, showed low MGI levels ranging from 6.7% to 2.1%, indicating that the nanoparticle alone was not effective in inhibiting fungal growth (Figure 2b). The regression coefficient for treatments was highly significant ($p < 0.05$), confirming their impact on mycelial growth inhibition. These results underscore the significant influence of the combination of Fludioxonil and Arbolina® on mycelial growth inhibition, while the experimental repetition had negligible impact.

Therefore, these findings indicate that, although fludioxonil exhibits intrinsic antifungal efficacy, its performance is substantially enhanced when incorporated into a nanoparticle-based delivery system.

4. Discussion

This study evaluated the potential of Arbolina® nanoparticles combined with fludioxonil to inhibit Monosporascus cannonballus growth in vitro. The nanoparticles were characterized by FTIR, UV-Vis spectroscopy, zeta potential, particle size analysis, and electron microscopy. The formulation enables controlled fungicide release, reducing required concentrations and targeting specific plant areas. Functionalized nanoparticles act as efficient carriers, ensuring targeted disease control without altering the fungicide's structure (Lima, 2020; Kumar et al. 2019).

Arbolina® nanoparticles showed absorption bands at 240 and 340 nm, linked to π–π* (C=C) and n–π* (C=O) transitions. Their π-conjugated structure and -NH2/-OH groups enable hydrophobic interactions and hydrogen bonding with fludioxonil, enhancing absorption and delivery efficiency (Maholiya et al. 2023; Zhao et al. 2021). Zeta potential (-17.8 mV) indicated high stability, while smaller particle size improved functionality and fluorescence (Sahu et al. 2012). TEM confirmed their morphology and role as effective carriers for controlled fungicide release.

While carbon dots are well-studied for antibacterial properties, their antifungal potential is less explored. This study suggests that the combination of Arbolina® nanoparticles with agrochemicals such as fludioxonil enhances stability and acts as a delivery and controlled release. The complete inhibition of *M. cannonballus* growth in nanoparticle-fungicide treatments in different concentrations highlights their role as carriers rather than active agents.

Finally, nanoparticles may enhance fungicide penetration and lower plant toxicity, while improving interactions with fungal cells. Similar findings with other nanoparticles, such as silver nanoparticles, support the potential of this approach for targeted, efficient pathogen control (Li et al. 2018; Almeida et al. 2021).

Conclusion

The study showed that Arbolina® nanoparticles enabled an efficient delivery in combination with fludioxonil *in vitro*. This approach enhances treatment effectiveness, allowing, perhaps, lower fungicide doses with stronger fungal control. Further studies are recommended to validate these findings and confirm the significant impact of treatments on mycelial growth inhibition, including field trials, while ensuring the robustness and reproducibility of the results across different experimental conditions. The nanoparticle combination with agrichemicals may offer an alternative and sustainable solution to reduce the environmental impact of chemical treatments, improving agricultural practices.

Declarations

FUNDING DECLARATION

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nanoparticle.

AUTHORS CONTRIBUTION

FRS, FAS carried out most Lab assays, collected part of the isolates, and wrote the first draft of the manuscript; PRVR and RCSC performed physicochemical characterization of samples and manuscript writing. CFF, FASA, RSJ and NFM contributed to the lab samples assays, and manuscript writing and reviewing; RCSC, FRS and FAS contributed to the Lab work and to the manuscript writing; FASA, RSJ and NFM contributed equally to the study conception. All authors read and approved the final version of the manuscript.

Data availability statement

The experimental and quantitative data from *Monosporascus cannonbalus* bioassays sampled in Brazil and associated data in this study will be available under request from the corresponding author NFM.

Ethics declaration: not applicable.

Consent to Participate declaration: not applicable.

Competing Interest declaration: 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.

References

1. Almeida A-SF, Corrêa Junior A, Bentes JLS (2021) Synthesis of silver nanoparticles (AgNPs) by Fusarium concolor and inhibition of plant pathogens. Summa Phytopathologica v 47(1):9–15
2. Brar SK, Verma M (2011) Measurement of nanoparticles by light-scattering techniques. TrAC Trends Anal Chem v 30(1):4–17
3. Bruton BD, García-Jimenez J, Armengol J (1999) Analysis of the relationship between temperature and vine declines caused by Acremonium cucurbitacearum and Monosporascus cannonballus on muskmelon. Subtropical Plant Sci 51:23–28
4. Egerton RF (2005) Physical Principles of Electron Microscopy: An Introduction to TEM, SEM and AEM. Springer Science + Business Media, Inc., New York, pp 125–153
5. Figueirêdo MCB, Gondim RS, Aragão FAS(org.) (eds) (2017) Produção de melão e mudanças climáticas: sistemas conservacionistas de cultivo para redução das pegadas de carbono e hídrica. Embrapa, Brasília, p 302
6. Instituto Brasileiro de Geografia e Estatística – IBGE (2020) SistemaIBGEdeRecuperaçãoAutomática-SIDRA.Disponívelem:https://www.ibge.gov.br/explica/producao-agropecuaria/melao/br.Acessoem:21mar.2022
7. Jing HH, Bardakci F, Akgöl S, Kusat K, Adnan M, Alam MJ, Gupta R, Sahreen S, Chen Y, Gopinath SCB (2023) Green Carbon Dots: Synthesis, Characterization, Properties and Biomedical Applications. J Funct Biomater 14:27. https://doi.org/10.3390/jfb14010027
8. Kumar S, Nehra M, Dilbaghi N, Marrazza G, Hassan AA, Kim KH (2019) Nano-based smart pesticide formulations: emerging opportunities for agriculture. J Controlled Release v 294:131–153
9. Kung J-C, Tseng I-T, Chien C-S, Lin S-H, Wang C-C, Shih C-J (2020) Microwave assisted synthesis of negative-charge carbon dots with potential antibacterial activity against multi-drug resistant bacteria. RSC Adv 10(67):41202–41208. 10.1039/d0ra07106d
10. LI H (2018) etal.Impacts of Carbon Dots on Rice Plants: Boosting the Growth and Improving the Disease Resistance. Materiais Biológicos Aplicados, v. 1, n. 3,p.663–672
11. Lima JD (2020) Desenvolvimentodenanoformulaçõesbiopesticidasaplicadasnaagricultura.48f.Dissertação(MestradoemCiênciadeMateriais)–UniversidadedeBrasília,Brasília,2020
12. Lin P-C, Lin S, Wang PC, Sridhar R (2014) Techniques for physicochemical characterization of nanomaterials. Biotechnol Adv v 32(4):711–726
13. Lowry GV, Hill RJ, Harper S, Rawle AF, Hendren CO, Klaessig F, Rumble J (2016) Guidance to improve the scientific value of zeta-potential measurements in nanoEHS. Environ Science: Nano 3(5):953–965. 10.1039/c6en00136j
14. Maholiya A, Ranjan P, Khan R, Murali S, Nainwal RC, Chauhan PS (2023) An insight into the role of carbon dots in the agriculture system: a review. Environ Sci Nano 10:959–995. 10.1039/d2en00954d
15. Manaia EB et al (2012) Liquid crystalline formulations containing modified surface TiO2 nanoparticles obtained by sol–gel process. J Solgel Sci Technol 63(2):251–257

16. Negreiros AMP et al (2019) Prevalent weeds collected from cucurbit fields in Northeastern Brazil reveal new species diversity in the genus Monosporascus. Annals Appl Biology v 174(3):349–363
17. Rodrigues M etal.Photosynthesis enhancer based on hybrid carbon nanoparticles, their production process and their use as nanobiostimulants and nanofertilizers in agricultural crops. CO2022012263A2. 2023-01-16 January 2023.Google patents,https://patents.google.com/patent/CO2022012263A2/en
18. Sahu S et al (2012) Simple one-step synthesis of highly luminescent carbon dots from orange juice: application as excellent bio-imaging agents. Chem Commun 48:8835–8837
19. SalesJúnior R et al (2018b) Podridão de raízes por Monosporascus e declínio de ramas no meloeiro: grave problema sem solução. In: LOPES UP, MICHEREFF SJ(org.) (eds) Desafios do Manejo de Doenças Radiculares Causadas por Fungos. EDUFRPE, Recife, pp 111–130
20. Shah MA, Wani SH (2016) Nanotechnology and insecticidal formulations. J Food Bioeng Nanoprocessing 1:285–310
21. ShahrzadRaeispour M (2024) AnanocarriersystembasedonCQDsforefficientmitoxantronedrugdelivery,Heliyon,Volume10,Issue11,2024,e31674,ISSN2405–8440,https://doi.org/10.1016/j.heliyon.2024.e31674
22. Vieira N, Simões W, Silva,José&Salviano,Alessandra&SoaresDaSilva,Jucicléia&Braga,Marcos&Guimaraes,Miguel&, Martins, Marcelo (2024) Cultivationofyellowmelonsubjectedtodifferentirrigationlevelsandapplicationofarbolina®biostimulant.RevistaCaatinga.37.10.1590/1983-21252024v3712452rc
23. Zhang H et al (2017) Filtration of Glycoprotein-Modified Carboxylated Polystyrene Microspheres as Cryptosporidium Oocysts Surrogates: Effects of Flow Rate, Alum, and Humic Acid. J Environ Eng v 143:1–9
24. Zhao S, Huang L, Xie Y, Wang B, Wang F, Lan M (2021) Green synthesis of multifunctional carbon dots for anti-cancer and anti-fungal applications. Chin J Chem Eng 37:97–104. https://doi.org/10.1016/j.cjche.2021

Figures

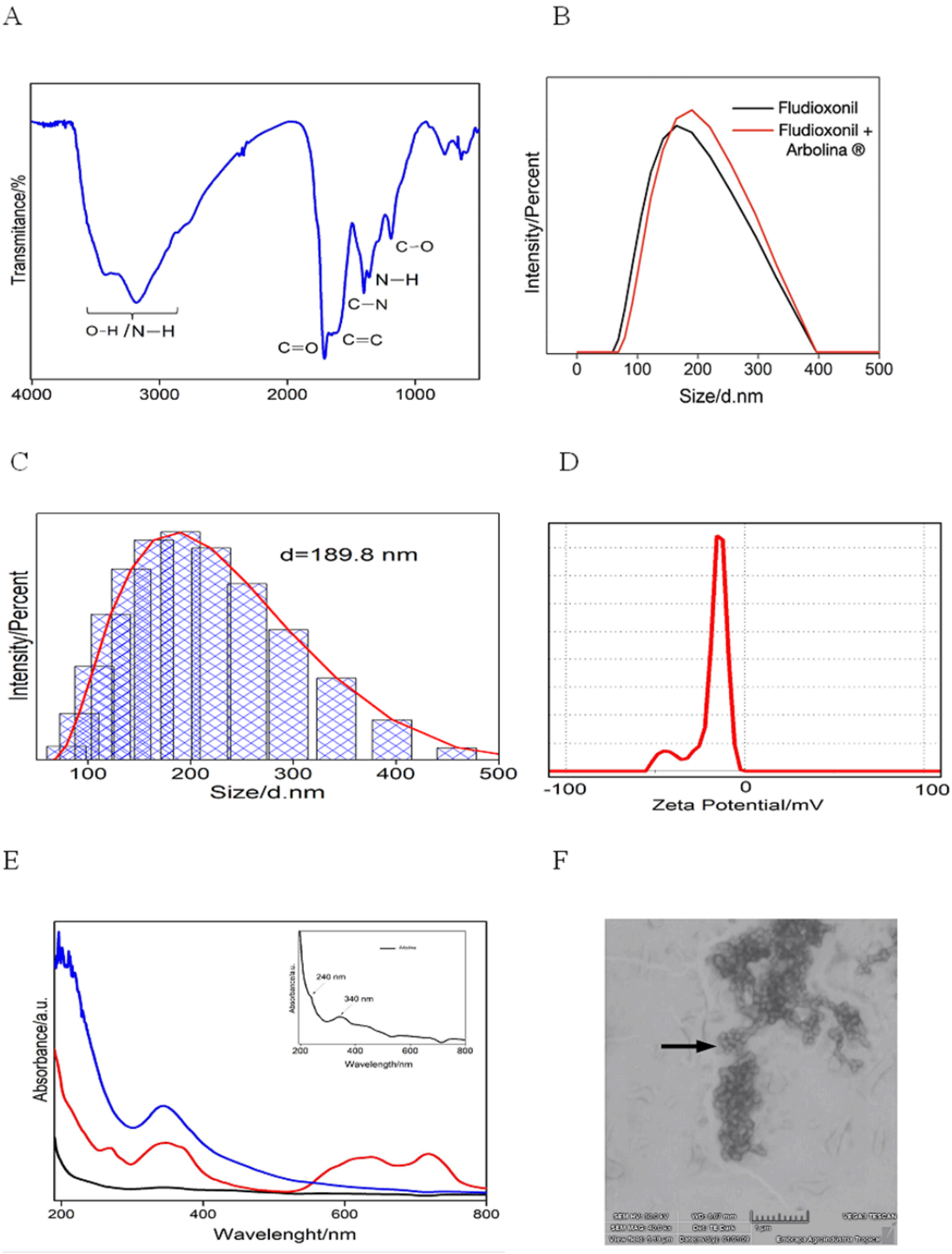
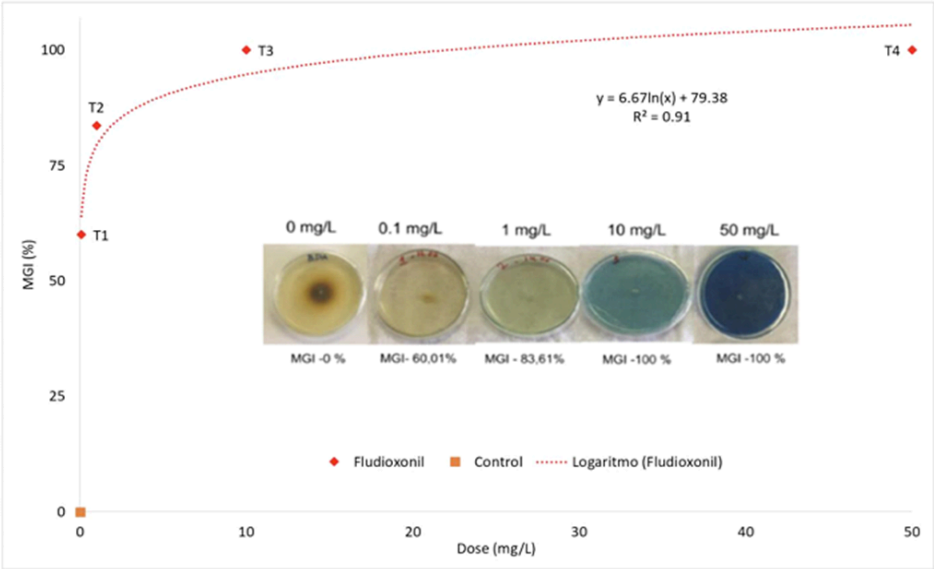


Figure 1

Nanoparticle Characterization. (A) Arbolina® FTIR spectrum and (B) Fludioxonil Size (black) and Arbolina® combined with fludioxonil (red). (C) Size deconvolution for Fludioxonil. (D) Zeta potential graph for Fludioxonil (E) Ovelaid UV-vis spectrum of Arbolina® (depicted), Fludioxonil (blue line) and Arbolina® combined with fludioxonil (red line) (F) Arbolina® HRTEM at 20 mg/L.

A



B

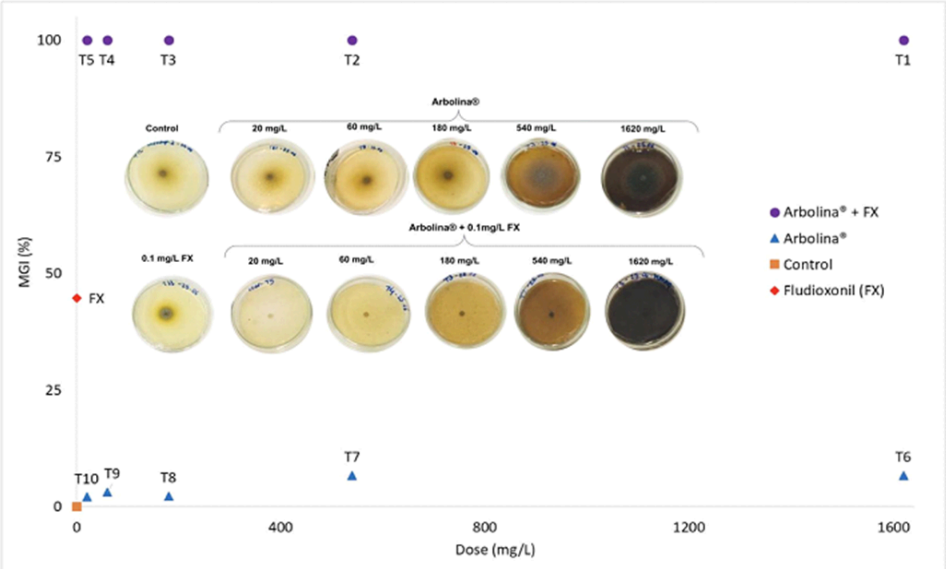


Figure 2

(a) Linear regression of dose-response bioassay of *M. cannonbalus* mycelial growth inhibition (MGI) under Fludioxonil from 0 to 50 mg/L. (b) Effect of Arbolina® Mycelial growth inhibition (%) of *Monosporascus cannonballus* in treatments of Arbolina® combined with Fludioxonil (FX).