Antifungal effect of silver nanoparticles on *Rickia wasmannii* infected ants

(Hymenoptera: Formicidae; Ascomycota: Laboulbeniales)

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Introduction

The aim of our research was to examine the possible antifungal effects of silver nanoparticles against *Rickia wasmannii* (Laboulbeniales, ascomycetes) infection on *Myrmica scabrinordis*. *Laboulbeniales* is a widely spread and diverse fungi class, with more than 2000 (sub)species, there are some agriculturally important insects amongst the possible infected host. According to our recent research *Rickia wasmannii* negatively affects the survivability and overall fitness of infected host ants.

Heavily infected *Myrmica scabrinordis* workers were separated from each other in special containers to avoid the possible cross-contamination and the experiments were carried out under the same environmental conditions.

Methods

Specimens

Myrmica scabrinodis workers have been collected from North-East Hungary, and due to the winter season they were kept in 4°C for the purpose of naturally occurring partial hibernation.

Preparation of samples

The experiment involved 7 ants. The ants were kept on 20 C° - after wakeup we slowly raised the temperature. We prepared special containers for the ants where they could acces their beverage without interaction with others, preventing cross contamination. The infected ants were treated with silver nanoparticle aerosols.

Figure 1. TEM picture of the silver colloid

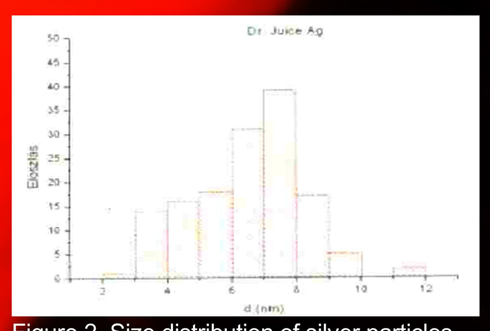


Figure 2. Size distribution of silver particles

Particle size analysis of the silver colloid used in the experiment

According to the literature, the silver colloid has antibacterial effect under 100nm, the effect getting stronger with the decrease of particle size, so we carried out an analysis confirming the exact size and purity of the colloid we used.
Based on the TEM picture in Figure 1. and the size distribution diagramm in Figure 2. it can be concluded that the colloid contains silver nanoparticles distributed around the size of 10nm.

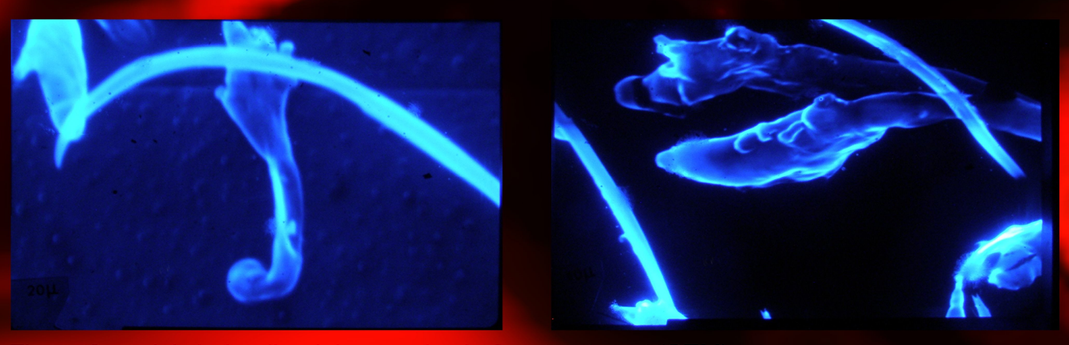


Figure 3. and 4. *Laboulbeniales* species before treatment (SEM)

Scanning Electron Microscopy

The infected ants were sprayed with silver colloid solutions of different concentrations, then incubated for seven days. Then the sample was kept in glutaraldehyde (4%) for 3 hours. After cleaning with destillated water the sample was dehidrated in etanol, dried and coated with gold. When the preparation was done the sample was scanned with Cambridge 604 Scanning Electron Microscope.

Stereomicroscopy

Post treatment pictures of the ants were captured with stereomicroscope on 32x and 50x magnification every day at the same time. The changes were detected and analysed quantitatively.Two rounds of experiments have been carried out, and the results were compared.In the analysis we measured hypha mortality on the abdomen of the ants.

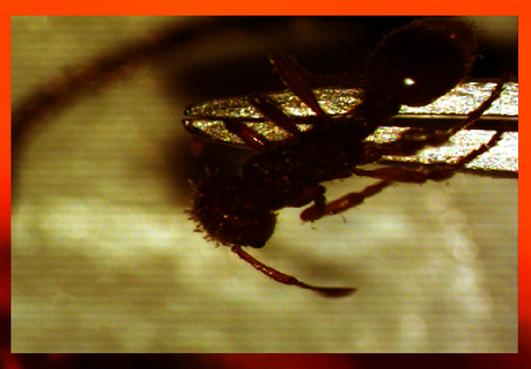


Figure 5. Infected control(*Myrmica scabrinodis* worker)

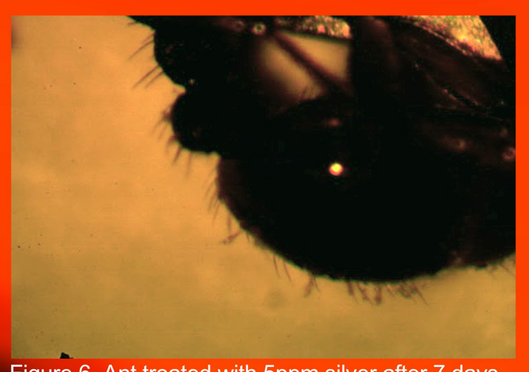
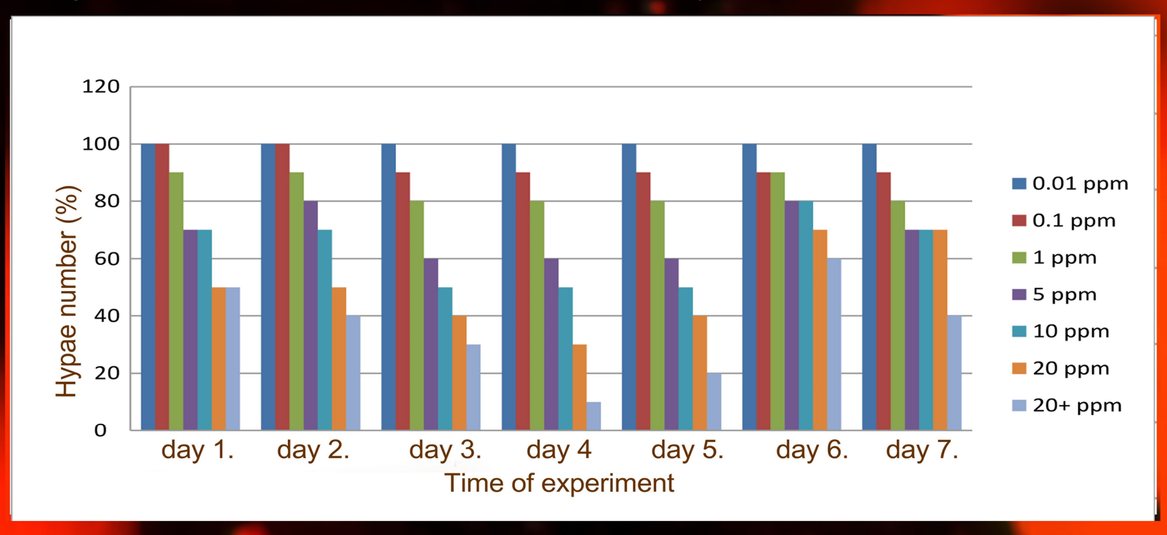


Figure 6. Ant treated with 5ppm silver after 7 days

Results

Our results show that lhe lowest concentrations (0.1-1 ppm) have no visible effect on the ants or fungi. The ideal concentrations (1-5 ppm) caused quantitative changes in the infection rate, as lowered the number of fungus hyphae in the infected regions against control.
In the future we would like to develop a protocol to clean the *Laboulbeniales* caused fungal infection from the agriculturally important insects.

Figure 7. The effect of silver colloid on the mortality of a *Laboulbeniales sp.*



The negative effects of colloid on the ants

Throughout the experiments several ants developed the condition of argyria. According to our experience the high concentration(20-20+ppm) silver colloid treatment results in argyria and leads to the death of the host.

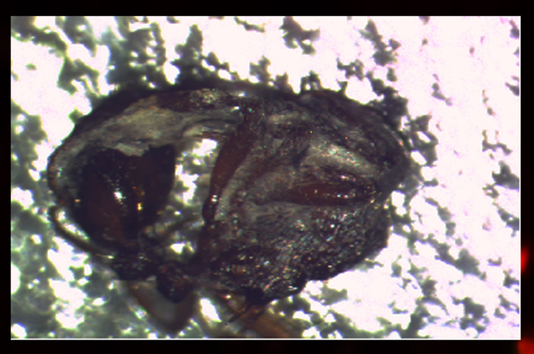


Figure 8. and 9. Argyria on *Laboulbeniales* infected *Myrmica scabrinordis*

Summary

The rapid specification of microbes poses a great threat to the agriculturally important insect species. *Laboulbeniales* species may become significant pathogens resulting in the early eradication of insect colonies. The in situ use of silver colloid may be a tool for the protection of insects.
Our results suggest that the use of silver nanoparticles in the eradication of pathogens of agriculturally important insect species might be effective, but many parameters will require investigation through further experiments and research, including toxicity and antimycotic effects in situ and development of methods to deliver the nanoparticles into pathogen colonized host tissues.

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