Fungal growth in response to various heavy metal concentrations

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Introduction

Heavy metals can either be beneficial or detrimental to fungal growth. This phenomenon, described as hormesis, depends on the metal type, concentration, fungal species, and heavy metal tolerance (Morkunas et al., 2018). As the natural environment becomes more contaminated by pollutants, investigating the effects of how heavy metals impact the soil microflora is increasingly important. In many habitats, fungi play a crucial role in the availability of nutrients and physiological processes for plant agriculture.

Previous research suggests that high levels of heavy metals inhibit enzymes and induce oxidative stress in fungi, whereas low concentrations may be necessary for metabolic processes (Anahid et al., 2011). Pathogenic and non-pathogenic fungi have unique metabolisms and environmental impacts; however, there is limited research on their responses to heavy metals. To address this, our study looked at *Sclerotinia sclerotiorum* Pers.: Fr, a pathogenic white-riot fungi (Kredics et al., 2001). In addition we also used *Trichoderma viride* (Lib.) de Bary, an antagonist to *Sclerotinia* spp. (Jaworska and Dłużniewska, 2007). Our focus on these two distinct organisms provides results that can be applied to many fungal species.

The objective of this experiment was to investigate the effects of heavy metals on the growth of fungi. We hypothesized that there would be a difference in the growth of *Trichoderma viride* and *Sclerotinia sclerotiorum* in response to different concentrations of $AlCl_3$ and $MnCl_2$. It was predicted that the growth of both species of fungi will be largest at 3mM.

Methods

Three stock fungal plates of each *Trichoderma viride* and *Sclerotinia sclerotiorum* were prepared on potato dextrose agar (PDA) plates prior to inoculation. PDA test plates were marked into trisects using the J Nowak template then grouped in triplets according to the heavy metal treatments: 0mM (control), 1mM, 3mM and 5mM of $AlCl_3$ and $MnCl_2$.

Fungal plugs were obtained from stock plates under UV light to minimize airborne spore transfer. Fungal plugs were randomly selected from the stock plate and inoculated to the appropriate treatment plate. Three plugs of each fungi were inoculated on each test plate; one treatment plug represented one replicate. Each heavy metal concentration resulted in a total of nine replications per species.

Once inoculated, plates were incubated in the dark for 5 days at 25ºC. After a full incubation period, the colony diameter (mm) of each replication plug was measured twice at perpendicular directions of each trisect.

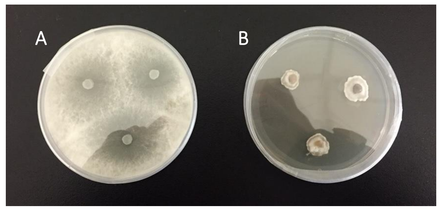

Figure 1. Two plates on the fifth day of incubation after being inoculated with (a) *T. viride* in 1mM of $AlCl_3$ and (b) *S. sclerotiorum* in 1mM of $MnCl_2$.

Statistical Analyses

One-way analysis of variance (ANOVA) was performed on colony diameter measurements and subsequently analyzed by Tukey's HSD test for pairwise comparisons of means (IBM SPSS Statistics 25 (2017)).

Results

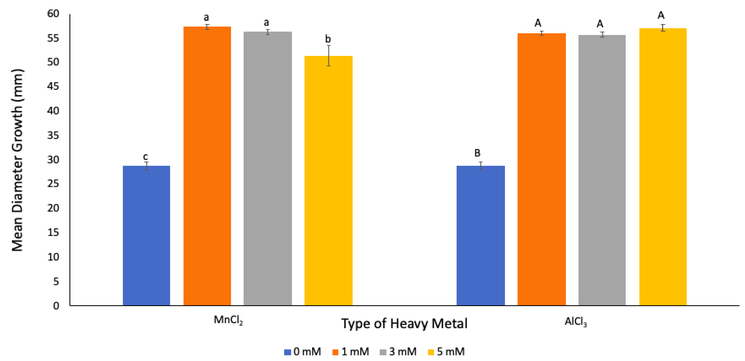

Figure 2. Mean ± SE diameter growth of *Trichoderma viride* at various concentrations of heavy metals. Means followed by the same letter within each response variable are not significantly different (P<0.05) according to Tukey's HSD test.

Figure 2 shows that fungal growth was generally higher in the presence of both heavy metals than the control. The mean diameter growth in 1 mM and 3 mM of $MnCl_2$ was significantly higher (F=132.87, P<0.001) than 0 mM and 5 mM. 1mM of $MnCl_2$ was the concentration with the greatest mean diameter growth, although not significantly different from the other concentrations. The mean diameter growth in all concentrations of $AlCl_3$ was significantly higher (F=498.25, P<0.001) than the 0 mM. The concentration of $AlCl_3$ with the greatest mean diameter growth, although not significantly different from other concentrations, was 5 mM. Overall, there is no visible trend in the growth of *T. viride* in the presence of both heavy metals (Fig. 2).

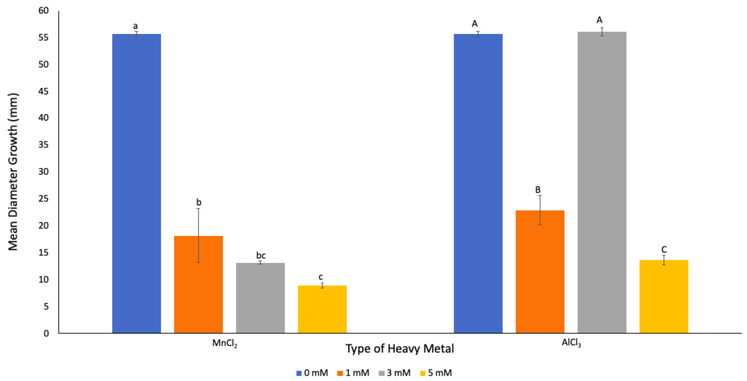

Figure 3. Mean ± SE diameter growth of *Sclerotinia sclerotiorum* at various concentrations of heavy metals. Means followed by the same letter within each response variable are not significantly different (P<0.05) according to Tukey's HSD test.

In general, Figure 3 shows that as the concentration of $MnCl_2$ increases, there was a decrease in growth. No general trend was observed for different concentrations of $AlCl_3$.The mean diameter growth in 0 mM of $MnCl_2$ was significantly higher (F=70.69, P<0.001) than the other concentrations of $MnCl_2$. The mean diameter growth in 0 mM and 3 mM of $AlCl_3$ was significantly higher (F=498.25, P<0.001) than the 1 mM and 5 mM. The concentration of $AlCl_3$ with the greatest mean diameter growth, although not significantly different, was 3 mM (Fig. 3).

Discussion

Both treatments of $AlCl_3$ and $MnCl_2$ influenced the growth of *S. sclerotiorum* and *T. viride,* supporting our hypothesis. Similar to our prediction, *S. sclerotiorum* growth was greatest at 3 mM $AlCl_3$ (Fig. 3). Increased growth at this concentration was not observed in *S. sclerotiorum* in response to $MnCl_2$ (Fig. 3) or in *T.viride* for both $AlCl_3$ and $MnCl_2$ treatments (Fig. 2). Our results suggest specific heavy metal concentrations can stimulate or inhibit fungal growth dependent on species and metal type.

Jaworska and Dłużniewska (2007) find that *T. viride* growth is unaffected in response to increasing Mn concentrations, which differs from our results. This discrepancy can be attributed to the different forms and concentrations of manganese used in the treatments. Aluminum provided a significant growth benefit to *T. viride* colonies (Fig. 2). Given the growth inhibition of *S. sclerotiorum* in response to aluminum (Fig. 3), *T. viride* may have unique tolerance to this heavy metal. This tolerance may involve extracellular sequestration through chelation or cell wall binding (Anahid et al., 2011). These mechanisms regulate the entry of heavy metals and control their effects in the cytosol (Baldrian, 2003; Bellion et al., 2006). Previous studies have shown that manganese is neither toxic to white-rot fungi nor is it accumulated in their fruit bodies (Baldrian, 2003). However, all concentrations of manganese in our experiment inhibited the growth of *S. sclerotiorum* (Fig. 3). In nature, the low concentrations of manganese found within the wood *S. sclerotiorum* inhabits may prevent heavy metal accumulation leading to toxicity (Baldrian, 2003).

Growth benefits provided to *T. viride* by manganese and aluminum could be used as an agricultural method to increase antagonism against plant pathogens such as *S. sclerotiorum*. As demonstrated by our study, appropriate concentrations of the same metal limits growth of *S. sclerotiorum* and could ultimately prevent white mold disease in plants.

It is reasonable to question the implications of anthropogenically-sourced heavy metals on symbiotic relationships between fungi and their surrounding environment. As discussed by Morkunas et al. (2018), the increase in heavy metal pollutant mobility can be attributed to climate change. Heavy metals that are accumulated in soils, or taken up by fungi, can quickly ascend the food chain leading to negative consequences (Morkunas et al., 2018).

In conclusion, our results suggested that various heavy metal concentrations have different influences on the growth of *S. sclerotiorum* and *T. viride*. The context of our study can be applied to larger-scale environmental concerns that could be examined in the near future. Many fungi can uptake more than one type of heavy metal and simultaneously interact with other surrounding fungi. Future studies could investigate the effects of various combinations of heavy metals, gaining more insight into hormetic responses and tolerance mechanisms in various fungal species.

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