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RESEARCH PAPER

Arbuscular Mycorrhizal Fungi Prevent Mercury Toxicity in *Lactuca sativa* (L.) Seed Germination

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Abstract
Mercury pollution is an issue of global concern. In Colombia, the use of contaminated water for food crop irrigation and artisanal mining contributes to mercury pollution in soil, affecting food production and restoration of disturbed areas. Mycorrhizal fungi are symbionts that provide benefits to plants including resistance to heavy metals, but fungal effects on germination remain to be fully described. This study tested the effect of mercury and mycorrhizal fungi on *Lactuca sativa* seed germination. A 2x5 completely randomized factorial experiment was developed to assess the effect of five $HgCl_2$ polluted treatments, two mycorrhizal treatments (i.e., with inoculum, without inoculum), and the interaction of both factors on seed germination, seedling root colonization, pH, and final water content. In samples with no mercury pollution, mycorrhizal fungi had an inhibitory effect on seed germination. Likewise, the effect of mercury on seed germination is significantly inhibitory. However, pots inoculated with arbuscular mycorrhizal fungi showed constant germination probabilities, independently of mercury concentration. According to the best model determined for the data, a key step in the mitigation of mercury toxicity in seed germination is to prevent substrate pH changes. The environmental conditions of the experiment contributed to densely activate populated biomass of inoculum, which promoted root invasion from various points. Overall, the presence of mycorrhizal fungi in seedbeds could lead to a reduced number of plant individuals. However, the use of fungal inoculum in polluted environments, highly contributes to plant establishment, which is relevant in further vegetable cultivations growing in soil polluted areas.

Keywords: Lettuce; post-germination; pollution; symbiosis.

INTRODUCTION

Anthropogenic pollutants are a major concern worldwide, due to their disruptive effect on ecosystem services provided by water bodies (Gerbersdorf et al., 2011). Wastewaters are commonly used in land irrigation for food production (Ye et al., 2018). The use of wastewaters in irrigation leads to elevated levels of heavy metals in soils and edible parts of food crops (Khan et al., 2008). In Colombia, the use of polluted water for food crop irrigation is common in places such as the Bogota river basin, where heavy metal accumulation was observed in lettuce (*Lactuca sativa* L.) and celery (*Apium graveolens* L.) (Miranda et al., 2008). Soil pollution by heavy metals is not only driven by artificial irrigation (Aasma et al., 2020). For instance, in

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Colombia, artisanal and small-scale gold mining pollutes water and sediments with mercury, which are then discharged into the soil during seasonal flooding (Marrugo-Negrete et al., 2010). Mercury exists in various chemical forms in polluted environments (Marrugo-Negrete et al., 2015; Pinedo-Hernández et al., 2015; Tiodar et al., 2021), promoting its bioaccumulation across the trophic net (Marrugo-Negrete et al., 2010). Consequently, plant growing in many regions of Colombia occurs in mercury-polluted environments.

Mercury can penetrate plants through the roots or leaves (Patra & Sharma, 2000). The most evident effect caused by mercury pollution is the reduction of plant size. High mercury levels in soil have been shown to gradually decrease the biomass of seedlings of several species, including *Mentha avensis* (Manikandan et al., 2015), *Cucumis sativus* (Cargnelutti et al., 2006), *Vigna radiata* (Zafar Iqbql et al., 2015), among others. This phenomenon is attributed to direct effects, such as the production of reactive oxygen species (ROS) in tissues where mercury is accumulated (Cargnelutti et al., 2006; Lomonte et al., 2010; Vargas-Aguirre et al., 2018), as well as indirect effects, such as limitation of mineral and water uptake (Manikandan et al., 2015) or carbon fixation (Patra & Sharma, 2000; Cargnelutti et al., 2006; Zafar Iqbql et al., 2015). Experiments on *V. radiata* showed that increased substrate contamination with mercury negatively affected seed germination, probably due to water uptake disturbance (Zafar Iqbql et al., 2015).

Plants exhibit strategies to overcome stress caused by heavy metals, such as the synthesis of antioxidant peptides (Patra & Sharma, 2000; Cargnelutti et al., 2006). Other responses to stress are ecological. These include the mycorrhizal symbiosis (MS), which is one of the most common ecological relationships on earth, where plant, fungal and bacterial partners establish complex interactions in the rhizosphere (Sánchez-Ramírez, 2017; Leudo et al. 2020, Ferreira & Oliveira, 2021). In MS, the fungal partner improves plant nutrient uptake, constituting the most efficient pathway for plants to acquire Phosphorus (P) (Perner et al., 2007; Ven et al., 2019). In exchange, the fungal partner acquires Carbon (C) delivered by plant roots as lipids (Luginbuehl et al., 2017). The most widespread MS is established between plants and arbuscular mycorrhizal fungi (AMF). AMF comprise a monophyletic group with low species diversity (Schüßler et al., 2001; Redecker et al., 2013) that exhibits obligate symbiosis (Smith & Read, 2008). It is proposed that, due to the early origin and central role of AMF during terrestrial plant evolution, these fungi may colonize the underground organs of all land plants (Smith & Read, 2008). MS confers other benefits to plants besides nutrient uptake, such as induction of tolerance to stress caused by drought (Chitarra et al., 2016), pathogens (Smith & Read, 2008), or heavy metal pollution (Hildebrandt et al., 2007; Yang et al., 2016; Vargas-Aguirre et al., 2018).

It is widely considered that seeds and germination are not affected by MS since AMF invades plant roots. Taking into account the need for strategies to promote the environmental recovery of zones affected by gold mining, and the fact that several food crops are located in soils polluted by heavy metals, it is necessary to consider the possible use of AMF as enhancers of germination that favor plant colonization in mercury-polluted landscapes. To assess this, an experiment was conducted to determine whether AMF can promote germination in a pollution-sensitive species under high levels of mercury in substrates. Lettuce was selected as study species since there is evidence of heavy metal accumulation in polluted soils used for lettuce cropping in Colombia (Miranda et al., 2008). Moreover, there is a long history of dose-response studies on lettuce in ecotoxicological contexts (e.g., Cozzolino et al., 2016). The main objective of this work was to assess if AMF can reduce mercury toxicity in lettuce germination.

MATERIALS AND METHODS

"Batavia" lettuce *Lactuca sativa* L. (Asteraceae) seeds produced by IMPULSEMILLAS (*Parque Industrial Gran Sabana*, Tocancipá, Cundinamarca, Colombia) were purchased from a local

distributor (Agrotienda El Ruiz, Manizales, Caldas, Colombia). The seeds were washed with sodium hypochlorite (0.75% v/v) and rinsed several times with distilled water. The disinfected material was air-dried for four hours and then deposited into paper packages containing 20 seeds each, where they were stored until planting in pots. The fungal inoculum used was of commercial type, named MICORRIZAS, purchased from a local distributor (ABONAMOS SA, Medellín, Colombia). This biofertilizer comprises approximately 365 infective propagules per gram, including colonized roots and viable spores and hyphae belonging to four AMF species: *Glomus fistulosum*, *Glomus manihotis*, *Entrophospora colombiana*, and *Scutellospora heterogama* (information supplied by the producer).

The substrate used was composed of peat and river sand. The former comprised *Sphagnum* (70%), fine vermiculite, macronutrients, lime, and wetting agents, and was purchased under the commercial name Pro-Mix PGX (Delson, Canada). This substrate has the following properties: pH (soil: water 1:5) 5.3, 700 g/kg organic matter, 70 mg/kg total N, 10 mg/kg total P, 55 mg/kg available K, 30 mg/kg Ca, 32.5 mg/kg Mg, and 1.4 mg/kg Fe. Peat was sieved using 500 μm-mesh sieves and then mixed with river sand in a 1:1 (v/v) proportion. The sand was treated to select only material between 500 and 45 μm through dry sieving; then, it was vigorously washed with tap water and air-dried. The substrate was divided into 2.5 kg bulks and autoclaved at 103.42 KPa and 121 °C for 12 h. Substrate charge capacity was 72.71% and the final pH was 5.3. Four flasks with sterile substrate were used to prepare the mercury polluted treatments by adding different dilutions of $HgCl_2$ in an aqueous solution to reach heavy metal concentrations of 1 mg/kg, 10 mg/kg, 100 mg/kg, and 1000 mg/kg in the dry substrate. These treatments were designed to assess the effect of highly toxic mercury concentrations on the symbiotic system. The fifth treatment was a control substrate without mercury. The substrates were oven-dried at 70°C until constant weight.

The mercury-amended substrates were divided into two groups. The first group was mixed with the biofertilizer MICORRIZAS at a final concentration of 15 propagules/g in the substrate (Varga, 2015) to obtain the mycorrhizal treatments (AMF). The second group was enriched with the same amount of previously autoclaved inoculum (i.e., at 103.42 kPa and 121 °C for 12 h) to conduct experimental controls without mycorrhizae (noAMF). The substrates were immediately hydrated to 70% water holding capacity and 230 ml of the prepared substrates were deposited in 236.5 ml polystyrene cups.

The methodology proposed by Vargas-Aguirre et al. (2018). The contents of the seed packages (i.e., 20 disinfected seeds each) were deposited in the substrates, and planting was done according to the manufacturer's instructions. A concave polystyrene piece was placed under each recipient to avoid mercury leaks during the experiment. The experiment was conducted in a greenhouse located in Manizales, Colombia (5°03'22.35" N, 75°29'41.72" W), at room temperature between 16 and 20 °C and relative humidity of 78%. A 2x5 completely randomized factorial design was used to assess the effect of five mercury polluted treatments and two AMF treatments (presence of mycorrhizae, AMF; absence of mycorrhizae, noAMF), and the interaction of both factors. Three replicates for each treatment combination were used (with 20 seeds per replicate). A total of 30 pots were established during the experiment.

All samples were harvested seven days after planting to assess the germination process before there were true leaves and seedlings were photosynthetically autonomous. This short time was used to ensure that there were no additional carbon sources for the MS other than the reserves stored in the seeds. The number of seedlings in each pot was determined. The root length of each seedling was measured. Substrate pH (soil: water 1: 5) was measured with a 210 Microprocessor pH Meter. Substrate water content was determined as the weight lost after drying at 70°C for 72 h until constant weight. Plant roots were fixed and stained with trypan blue (Phillips & Hayman, 1970), and root colonization percentage was measured using the intersect method (Giovannetti & Mosse, 1980).

In total, this study tested the effect of two symbiotic and five pollution levels on five variables: germination, seedling root length, pH, substrate humidity, and root colonization percentages. All statistical analyses were conducted in R 4.0.2. Data analysis was performed on each response variable, to assess the best model to analyze each type of data (Zuur et al., 2010). Boxplots representing each variable were generated using the function '*boxclust*' in the '*toxbox*' package (Pallmann & Hothorn, 2016).

Analysis of the AMF colonization in the different treatments showed a nonparametric pattern. After detection of differences through the Kruskal-Wallis test, the effects of $HgCl_2$ concentration on AMF colonization in seedling roots were assessed through Dunn's test by comparing root colonization at each concentration against the non-contaminated control using the '*nparcomp*' function (Konietschke et al., 2015).

The overall effect of the AMF was tested by comparing the germination in the noAMF and AMF pots. Residual analysis showed heteroscedasticity. Welch's heteroscedastic F test was used to compare germination between AMF inoculated and non-inoculated pots using the function '*welch.test*' in the '*onewaytests*' package (Dag et al., 2018). When grouped by mercury pollution, data showed a non-normal distribution. Kruskal-Wallis tested the existence of differences, and the Dunn's test was used as a *post hoc* procedure separately on AMF and noAMF data to compare germination in mercury treatments against the non-polluted substrate (Konietschke et al., 2015).To test the interaction of the effects of the fungi and the pollutant, germination data were analyzed using a generalized linear model with a binomial distribution, using fungal treatment (noAMF, AMF), pollution level ($HgCl_2$ concentration), and their interaction as fixed factors. An analysis of variance (ANOVA) was conducted with the '*Anova*' function in the 'Car' (Fox & Weisberg, 2019) package was conducted due to the normal distribution and homoscedasticity of the residues.

The effect of $HgCl_2$ and AMF on two important substrate characteristics that affect seed germination was tested: pH and water content. Because of normal distribution and homoscedasticity, *t.test* was used to compare pH among inoculated and non-inoculated pots. When grouped by pollution level, they showed to be nonparametric. Dunn's test (Konietschke et al., 2015) was used to verify if $HgCl_2$ concentration affected pH separately on AMF and noAMF pots. The final water content showed the same characteristics, so it was analyzed similarly.

To test whether substrate variables modified by AMF and mercury could explain changes in *L. sativa* seed germination probability, a linear mixed model was conducted by testing germination as a dependent variable; and pH, water content, and colonization as predictor variables; and inoculum and $HgCl_2$ concentration as random factors. The fusing function '*lme*' in the '*nlme*' package was used (Pinheiro et al., 2007). Mercury concentration was transformed by calculating the log of $HgCl_2$ concentration +0.1 before its inclusion into the analysis. The model was fitted by restricted maximum likelihood. Since regression-based models can be sensitive to correlated variables, variance inflation factors (VIF) for all predictor parameters in the model were calculated according to the standard protocols (Zuur et al., 2010). VIFs for all parameters, except for germination probability, fell below the common threshold value. Since germination probability is the dependent variable supported by the other variables included in the calculation, no parameters were excluded based on collinearity.

Finally, to assess the effect of the mycorrhizal fungi on germinated seedlings, the differences in root length among seedlings grown in AMF and noAMF pots were compared. These two sets of data proved to be heteroscedastic, so Welch's test was used as previously described. The effect of $HgCl_2$ concentration on seedling root length was tested separately in noAMF and AMF pots through a generalized linear model using the '*lm*' R function. Parametric statistics were used for this comparison. The model obtained was tested for significance using an analysis of variance conducted with the '*Anova*' function in the 'Car' package (Fox & Weisberg, 2019).

RESULTS AND DISCUSSION

Fungal structures were detected in seedling roots grown in AMF pots. Some root fragments in noAMF pots were detected as positive for fungal structures, but these were considered dye artifacts. No effect of $HgCl_2$ concentration was observed in AMF root colonization in seedlings from AMF pots (Fig. 1), yet greater variance was observed at the highest mercury concentration.

Significant effects of $HgCl_2$ concentration (p-value < 0.01) and AMF inoculum (p-value < 0.001) on germination were detected. Germination success was different in AMF and noAMF pots. In noAMF pots, germination probability was close to 0.8 (Fig. 2.A), although germination was reduced in pots with 1000 ppm $HgCl_2$ (Fig. 2.B). In AMF pots, germination probability was approximately 0.6 and showed low variance. Conversely, in noAMF pots, a higher variance was attributed to the low germination rate in highly polluted substrates. The difference in germination probability was statistically significant between noAMF and AMF substrates. This finding demonstrates a lower germination probability in AMF pots related to inoculum application, which reduces the number of successfully germinated seedlings.

Mercury affected lettuce seed germination in noAMF pots (Fig. 2.B). In absence of AMF, there were no changes in seed germination in response to mercury concentrations up to 100 mg/kg. However, at the highest concentration, germination probability was reduced to almost 20%. In contrast, in AMF substrates, germination remained constant at the different mercury concentrations tested and no significant differences were detected compared with the controls (i.e., without mercury) (Fig. 2.C). Therefore, no effect of mercury on germination success was observed when germination occurred in presence of AMF inoculum.

The AMF inoculum did not modify substrate pH (*t.test*, p-value = 0.253). Substrates in non-polluted treatments had a pH of 5.4 ± 0.19. However, pH was significantly altered at the highest mercury concentration in noAMF pots, demonstrating that 1000 mg/kg $HgCl_2$ promotes substrate acidity, indicated by a lower pH compared with the non-polluted control without AMF inoculum (Fig. 3.A). Different results were observed among pots with AMF inoculum. For

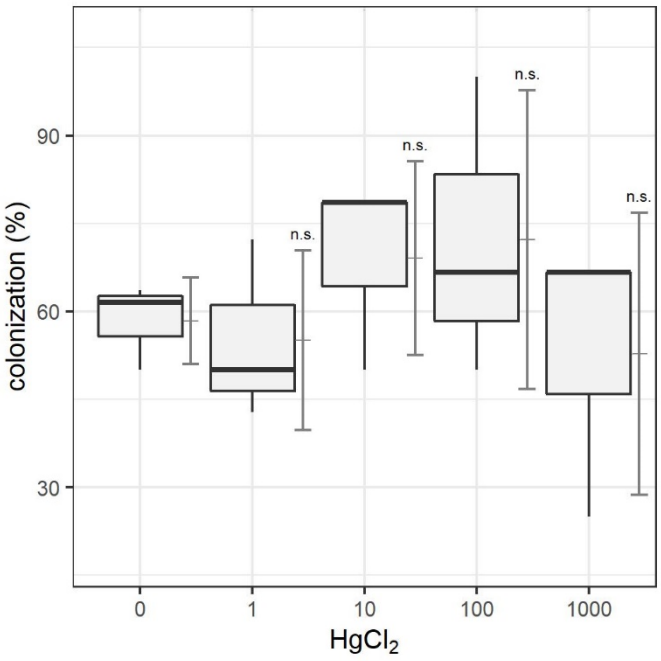

Fig. 1. Effect of $HgCl_2$ concentration on seedling root colonization by AMF. P-values of Dunn's comparison of each treatment with the control are shown

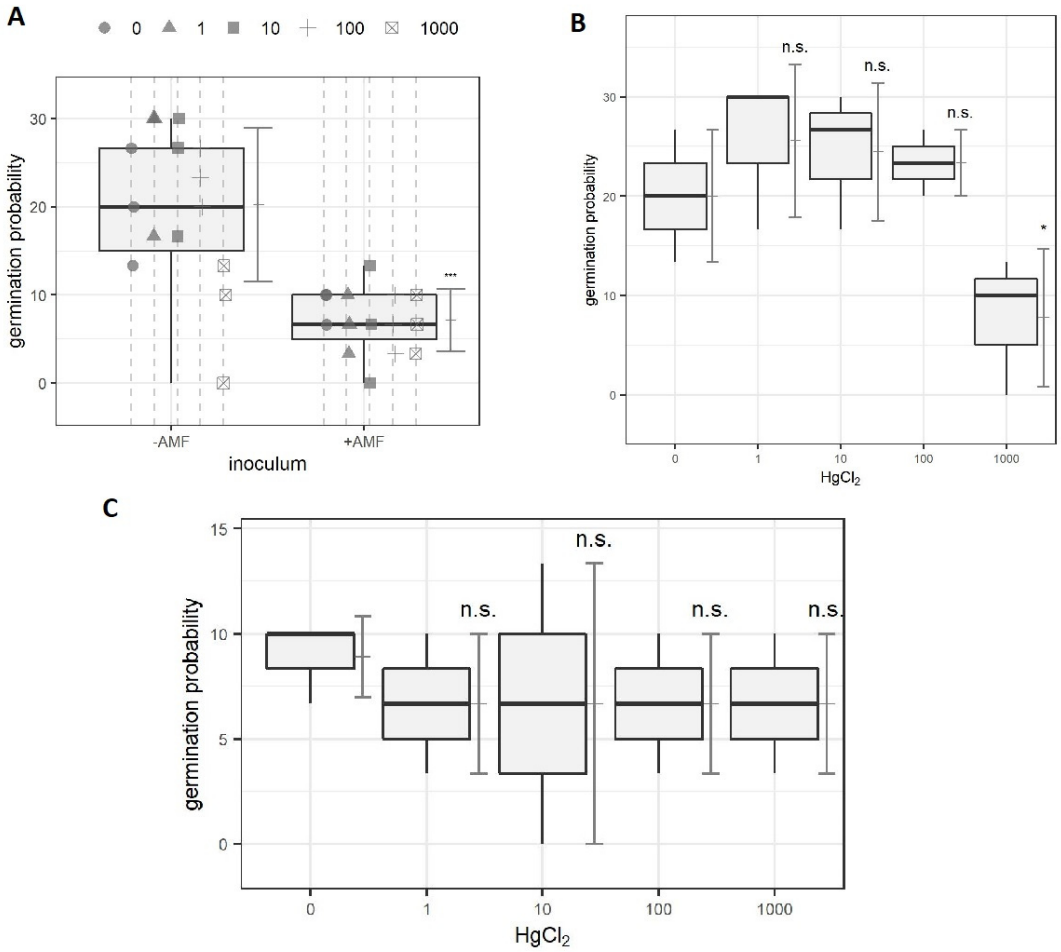

Fig. 2. a) Effect of AMF on *L. sativa* seed germination probability. Significant differences shown by Welch's heteroscedastic F test are indicated. The shapes indicate different $HgCl_2$ concentrations. b) Effect of mercury on *L. sativa* seed germination in noAMF and (c) AMF pots. P-values of Dunn's comparison of each treatment with the control without mercury are shown in (b) and (c). Significance codes: ns, p-value>0.05; *, p-value<0.05; **, p-value<0.01; ***, p-value<0.001

instance, pH remained constant across all mercury concentrations, yet the variance increased among treatments in response to $HgCl_2$ concentration (Fig. 3.B). Substrate water content did not show differences between AMF and noAMF pots (*t.test*, p-value = 0.271). Similarly, no significant differences in substrate water content among polluted and non-polluted treatments were observed in noAMF or AMF pots.

Water content was the only factor in the model that showed no significant effects on germination probability, yet the removal of this term turned all other parameters insignificant (Fig. 4.A and Fig. 4.B). $HgCl_2$ concentration, colonization, and pH appeared as significant terms driving *L. sativa* seed germination (Table 1). Germination exhibits a negative relationship with these terms.

The post-germinated seedlings were affected by AMF inoculum and mercury concentration (Fig. 5). Seedlings grown in AMF pots grew significant longer than those grown in noAMF pots (Fig. 5). A significative effect of $HgCl_2$ concentration on root length was detected in noAMF pots (p-value < 0.001). Reduced root length was observed in polluted pots without AMF. On the other hand, root length was not affected by $HgCl_2$ concentration in the substrate (p-value = 0.1935).

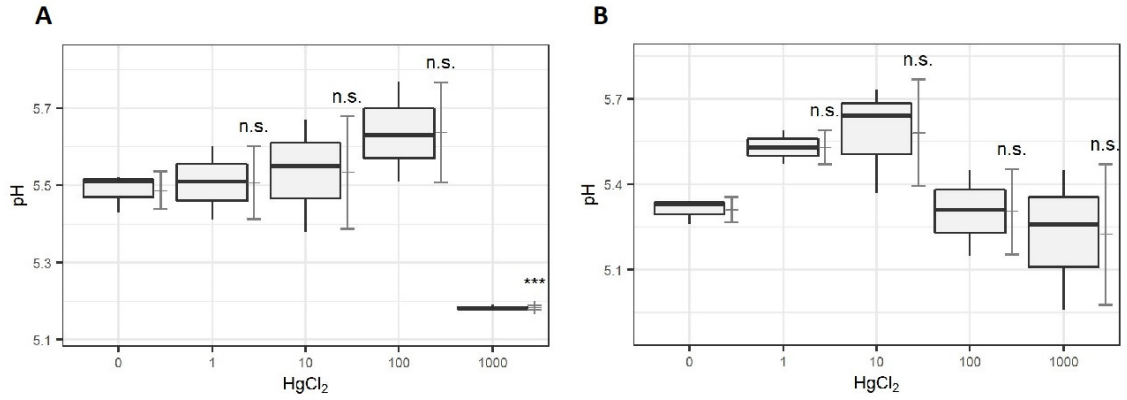

Fig. 3. Effect of mercury on pH in a) no AMF and (b) AMF pots. P-values of Dunn's comparison of each treatment with the control without mercury are shown. Significance codes: ns, p-value>0.05; *, p-value<0.05; **, p-value<0.01; ***, p-value<0.001

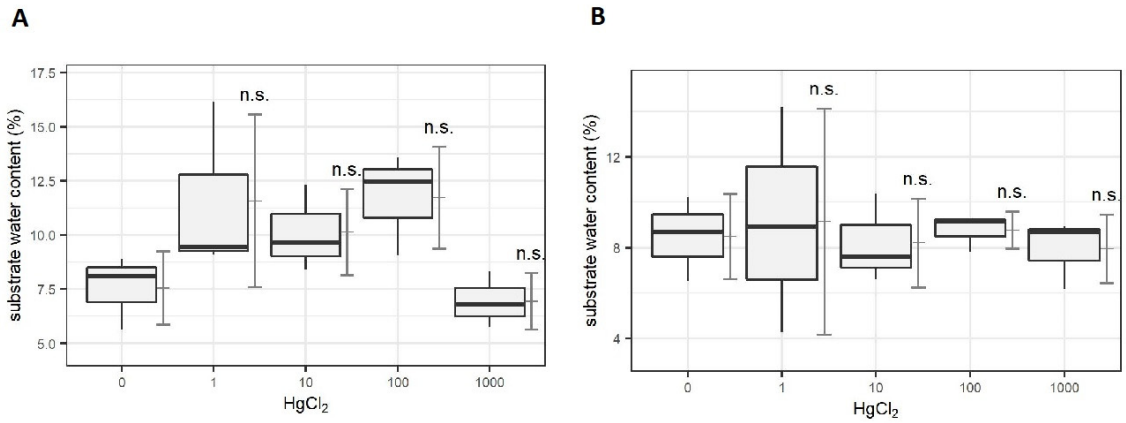

Fig. 4. Effect of mercury on substrate water content in (a) no AMF and (b) AMF pots. P-values of Dunn's comparison of each treatment with the control without mercury are shown. Significance codes: ns, p-value>0.05; *, p-value<0.05; **, p-value<0.01; ***, p-value<0.001

Table 1. Results of the linear mixed effects model.

Fixed Effects	Estimate	Standad Error	df	t value	Pr (>|t|)
pH	-4.490776	1.762424	6	-2.548068	0.0436
water content	-2.085652	0.919698	6	-2.267756	0.0639
colonization	-0.620952	0.223676	6	-2.776125	0.0322
log[Hg+0.1]	-5.909813	1.715776	7	-3.444396	0.0108

The establishment of a functional MS demands the development of complex structures by both fungi and plants (Smith & Read, 2008). This process has been divided into four stages: the presymbiotic stage, the hyphopodium formation stage, the pre-penetration apparatus formation stage, and the cortex invasion stage (Gutjahr & Parniske, 2019). The presymbiotic stage is defined by a fine exchange of molecular messages between plant and fungal partners, mediated by a complex mix of molecules (Gutjahr & Parniske, 2019). Among these molecules, the most important for AMF activation are strigolactones (SLs) (Xie et al., 2010; Gutjahr & Parniske,

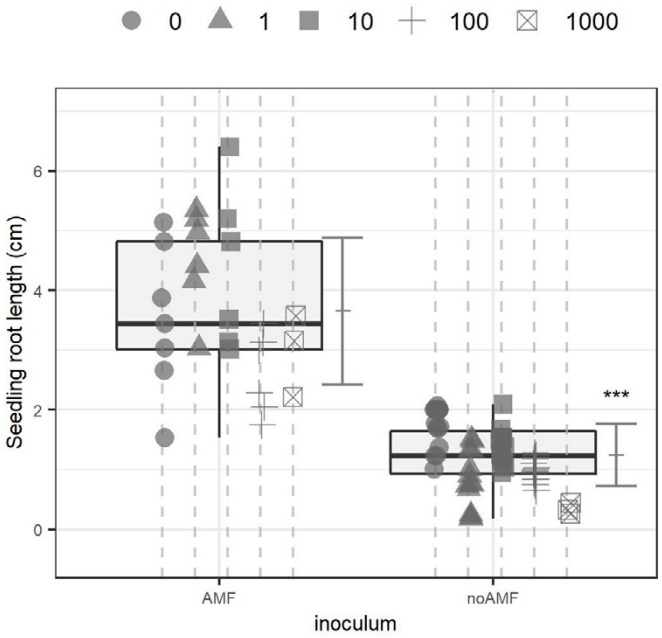

Fig. 5. Effect of AMF on the percentage of *L. sativa* seedling root colonization. Significant differences shown by Welch's heteroscedastic F test are indicated. The shapes indicate different $HgCl_2$ concentrations. Significance codes: ns, p-value>0.05; *, p-value<0.05; **, p-value<0.01; ***, p-value<0.001

2019), a class of plant hormones affecting plant development (Xie et al., 2010). SLs are signaling molecules produced by plants and released to the soil in root-exudates, thus, constituting critical cues for spore germination and hyphal elongation and branching (Gutjahr & Parniske, 2019). Once AMF hyphae elongate and establish contact with the roots, these fungi develop penetration structures that lead to the formation of the hyphopodium in the rhizodermis, a process guided by the physicochemical qualities of the root, such as the presence of cutin precursors (Gutjahr & Parniske, 2019). In this study, the roots developed by *L. sativa* seedlings were undergoing rapid growth and branching, which are processes regulated by SLs; furthermore, the synthesis of cutin precursors for rhizodermis could be considered high due to the rapid growth. Altogether, these conditions could activate a densely populated biomass of inoculum promoting root invasion from various points, thus, explaining the high colonization reported in the experiment.

SLs have a wide range of functions besides MS presymbiotic communication. SLs are a family of plant hormones involved in shoot branching, root architecture, photomorphogenesis, and seed germination (Gutjahr & Parniske, 2019). The levels of this phytohormone in roots are relatively high, whereas, in other tissues, such as the hypocotyl, stems, and leaves, SLs levels are low or undetectable (Ruyter-Spira et al., 2012). Although SLs were first described as promotors of seed germination in parasitic weeds (Xie et al., 2010; Ruyter-Spira et al., 2012; Gutjahr & Parniske, 2019), it has been shown that several synthetic SLs inhibit seed germination in various crop species, including *L. sativa* (Pepperman & Bradow, 1988). SLs can shed light on understanding the significantly lower seed germination probability of *L. sativa* in +AMF pots, one of the most striking results of our experiment. Furthermore, other reports show inhibitory effects of AMF inoculum on seed germination. For example, experiments with *Geranium sylvaticum* L. showed that the presence of AMF spores negatively affected seed germination in absence of a pre-established AMF hyphal network, and the inhibitory effect was attributed to root exudates (Varga, 2015). An inhibitory effect of AMF inoculum and preexisting AMF mycelium network

on germination in *Centaurea nigra* L. was also observed, which was attributed to changes caused by AMF in soil physicochemical properties or to the mobilization of plant allelochemicals through the hyphal network (Maighal et al., 2016).

Despite the reduced number of germinated individuals in AMF pots, there were evident benefits gained by *L. sativa* grown with AMF inoculum in polluted substrates. Mercury affected *L. sativa* germination in absence of AMF inoculum. Disruptive effects of $HgCl_2$ on *L. sativa* seedling morphohistology have been previously reported and such effects were attributed to oxidative stress caused by the heavy metal (Vargas-Aguirre et al., 2018). Indirect effects of mercury may also be involved in its impact on plant establishment, including changes in plant-water relations that affect water and mineral uptake (Patra & Sharma, 2000; Cargnelutti et al., 2006; Manikandan et al., 2015; Zafar Iqbql et al., 2015). Two variables are known to explain major variances in seed germination: osmotic pressure and pH (Dias et al., 2016). In this experiment, final water content remained constant and the substrates comprised the same organic material composition, so it is assumed that differences in osmotic pressure were negligible. pH was significantly more acidic at 1000 mg/kg $HgCl_2$ treatment in absence of AMF inoculum, which also showed a significant reduction in germination probability. Highly acidic pH has been reported to reduce or even inhibit *L. sativa* seed germination (Dias et al., 2016), so it is plausible that the direct effect of mercury as an oxidative-stress causing agent and the indirect effect of this heavy metal in substrate variables, such as pH, contribute to explain reduced germination probability in the highest mercury polluted noAMF treatment.

The effects of mercury on germination or pH were not observed in AMF pots at any $HgCl_2$ concentration. Evidence shows that AMF can filter out toxic heavy metals through several mechanisms that include fungal accumulation of heavy metals in cell walls, dense cytoplasmatic granules, or glomalin (Hildebrandt et al., 2007); thus, preventing contact of heavy metals with the plant. It has been shown that AMF can contribute directly to *L. sativa* seedling morphological integrity by improving nutritional conditions rather than preventing cell damage caused by mercury (Vargas-Aguirre et al., 2018). The data shown here indicate that AMF act as buffers against the effect of $HgCl_2$ on substrate pH, adding to their nutritional effects on host seedlings. Therefore, the presence of AMF prevents pH changes observed in substrates containing high mercury levels without AMF inoculum. Protecting against pH changes is likely a key step in preventing germination inhibition by $HgCl_2$ since pH is the second most important variable affecting the outcome of germination.

The experiments conducted here show contrasting facets of AMF. First, AMF may be an obstacle for plant establishment. Seeds in a cohort must cope with the AMF needs for carbon in the absence of a photosynthetic machinery successfully stablished. In this study, it is hypothesized that chemical communication between the first germinated seedlings and AMF can affect adjacent seeds in the same pot. However, this process has an overall beneficial outcome for successfully germinated plants in AMF pots, including mitigation of the effect of mercury on seed germination and faster growth in presence of AMF. Increased growth is one of the most well-known effects of AMF on plant physiology. In this assay, AMF significantly increased plant growth in the pre-photosynthetic stage, providing evidence for the effect of AMF on carbon use optimization by young plants, but also preventing negative effects of $HgCl_2$ on seedlings. Nutritional effects (Vargas-Aguirre et al., 2018), prevention of oxidative effects (García-Sánchez et al., 2014; Hildebrandt et al., 2007), and heavy metal immobilization (Hildebrandt et al., 2007) can altogether contribute to explain these results.

The generalization of these results should be made with caution. The reactivity of seed germination to AMF is not homogeneous, at least across plant-host species (Maighal et al., 2016). Here, an effect of AMF on a food crop is presented. Consequently, care should be taken in facilities devoted to the cultivation of species for commercial production or reforestation. The use of AMF in seedbeds could lead to a reduced number of individuals. However, the use of

AMF inoculum after transplantation could contribute to achieving better performance in plant establishment, especially in harsh environments.

CONCLUSION

Arbuscular mycorrhizal fungi reduce *L. sativa* seed germination; however, they also contribute to post-germinated seedling growth through several mechanisms. $HgCl_2$ modifies seed germination and post-germinated seedling growth when plants develop in absence of mycorrhizal fungi. However, in presence of AMF, germination and pre-photosynthetic growth of *L. sativa* remain unaltered since substrate conditions are unmodified. AMF inoculum can be an effective alternative to promote plant establishment after transplant to mercury polluted substrates.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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