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Effect of mycorrhizal inoculation on black and white poplar in a lead-polluted soil

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ABSTRACT: A pot experiment was carried out to examine the effect of inoculation with arbuscular mycorrhizal fungi (originated from a soil polluted with Pb) on root mycorrhizal colonization, survival, growth and volume production of black and white poplar plants grown on polluted (Pb) and non-polluted soils. In July, mycorrhizal inoculation had no significant effect on stem growth and volume production of black and white poplar plants grown on polluted and non-polluted soils. In October, mycorrhizal inoculation improved only parameters of white poplar plants (survival on polluted soil; growth and volume production on polluted and non-polluted soils). Mycorrhizal inoculation increased the root colonization percentage of black and white poplar plants on both soils. Generally, black poplar plants had significantly better survival, root mycorrhizal colonization, stem growth and volume production than white poplar plants. Although mycorrhizal and non-mycorrhizal plants of black poplar on polluted soil had higher survival, growth and volume production than white poplar, however in white poplar mycorrhizal inoculation improved the efficiency of plants on polluted soil.

Keywords: arbuscular mycorrhizal fungi; contaminated soil; lead; phytoremediation; *Populus*

High amounts of heavy metals in soils originate from human activities such as burning of fossil fuels, use of mineral fertilizers, release of wastes, mining and etc. (Yanqun et al. 2004; Abdullahi et al. 2009). Some heavy metals (Zn, Cu, Mn, Fe and Ni) are essential elements for metabolic processes of plants, but on the contrary, other heavy metals (Cd, Pb and Hg) are not necessary for plants (Rafati et al. 2011). The sensitivity of plant species to the heavy metal lead (Pb) is different (Arriagada et al. 2005). Generally, toxic concentrations of Pb are described as 30–300 mg·kg^{-1} dry weight (Baycu et al. 2006).

In general, traditional remediation methods of soil are costly and harmful to soil properties (Mulligan et al. 2001). Therefore, phytoremediation of soils polluted with heavy metals, which employs plants for this purpose, has received much consideration as a low-cost and environmentally friendly method (Komarek et al. 2007; He et al. 2013). The majority of phytoremediation surveys are focused on the use of fast-growing plants with high biomass yields. Tree species belonging to the family Salicaceae are attended as suitable candidates for phytoremediation of soils polluted with heavy metals due to fast growth, metal tolerance and ability to accumulate heavy metals (Pulford, Dickinson 2005).

Soil microorganisms have a significant impact in plant tolerance to heavy metal stress (Kabata-

Pendias 2004). The arbuscular mycorrhizal fungi (AMF) are a main portion of soil microbial biomass (Arriagada et al. 2005). Under natural conditions, AMF create symbiotic associations with the roots of many plants (Smith, Read 1997). AMF symbiosis was reported by Khasa et al. (2002) and Lingua et al. (2008) also in young poplars. In fact, the association of plant roots with AMF could improve plant growth and phytoremediation efficiency (Bissonnette et al. 2010).

Successful establishment of plants in soils polluted with heavy metals is an important factor in the phytoremediation process (Zalesny et al. 2005). The aim of the present study is to survey the potential establishment of black poplar (*Populus nigra* Linnaeus clone 62/154) and white poplar (*Populus alba* Linnaeus clone 44/9) in Pb-polluted soil, when inoculated with a fungal community obtained from a Pb-polluted soil. Our goals were (*i*) to investigate the influence of mycorrhizal inoculation on root mycorrhizal colonization, survival, growth and volume production of poplar trees in Pb-polluted soil, (*ii*) to compare the performance of poplars under tested treatments.

MATERIAL AND METHODS

In this study, poplar plants were grown from cuttings collected from the nursery of Research Institute of Forests and Rangelands in Karaj, Iran. We selected *P. nigra* 62/154 and *P. alba* 44/9 clones as these clones are commonly used in Iranian poplar plantations. The cuttings were gathered in February and maintained at 4°C until planting date.

We used an arbuscular mycorrhizal (AM) fungal community originated from a soil contaminated with 600 mg Pb·kg^{-1} for inoculation. We identified 9 AMF on the species level in the AM fungal community: *Glomus geosporum*, *G. intraradices*, *G. mosseae*, *G. fasciculatum*, *G. caledonium*, *G. clarum*, *G. constrictum*, *G. aggregatum* and *G. drummondi*. To produce fungal inoculum, the above-mentioned fungal community was propagated on maize (*Zea mays* Linnaeus) for 4 months on a sterilized soil (Chellappan et al. 2002). At the same time, the non-mycorrhizal inoculum was produced with the similar sterilized substrate on which maize was grown. Finally, the fungal inoculum was a mix of mycorrhizal root fragments of *Z. mays* and soil with fungal hyphae and spores. In contrast, non-mycorrhizal inoculum was a mix of non-mycorrhizal maize roots and soil without AM fungal propagules. On average, there were 5 fungal spores per 1 g fungal inoculum.

Table 1. Physical and chemical properties of the soil used for the experiment

Sand (%)	46.5
Silt (%)	34.2
Clay (%)	19.3
Soil texture	sandy-loam
pH (in water)	7.4
EC (ds·m^{-1})	0.95
CEC (meq·100 g)	14.3
Organic matter (%)	1.3
Total N (%)	0.16
Assimilable P (mg·kg^{-1})	10.8
Exchangeable K (mg·kg^{-1})	105

EC – electrical conductivity, CEC – cation-exchange capacity

In late March, the cuttings were removed from cold storage and left overnight under running tap water. In each 7-l pot, one cutting was planted and the fungal inoculum (100 g consisting of roughly 500 spores) was spread around each cutting. For control treatment, the same dose of non-mycorrhizal inoculum was used. Soil properties are indicated in Table 1. A part of the prepared soil was polluted with $Pb(NO_3)_2$ equal to 1000 mg·kg^{-1}. The pots were kept in a greenhouse under natural light and at a temperature between 15 and 25°C about 6 months and irrigated as necessary.

The pot experiment was a factorial completely randomized block design, with 2 factors: (*i*) AMF in 2 levels (non-mycorrhizal and mycorrhizal inoculation), (*ii*) soil in 2 levels (non-polluted and polluted soil). Per each poplar clone we had 4 treatments (2 fungal treatments × 2 soil treatments), with 12 plants in each treatment.

Survival and stem growth (stem length (SL) and stem basal diameter (SD)) of plants were measured in the middle and at the end of the growing season (July and October). Volume production was computed by the generalized equation volume = diameter^2 × height (Avery, Burkhart 1994). Also, for determination of the root colonization percentage with AMF, clearing and staining of roots were done using the standard method of Phillips and Hayman (1970), and then the root colonization percentage was determined by the gridline intersect method (Giovannetti, Mosse 1980).

To compare root mycorrhizal colonization, survival, growth and volume production of non-mycorrhizal and mycorrhizal plants of each clone and also plant data between two clones, we used *t*-test ($P < 0.05$). Statistical analysis was conducted using SPSS software (SPSS, Inc., Chicago, USA).

RESULTS AND DISCUSSION

On polluted and non-polluted soils and in the middle and at the end of the growing season (July and October), the root mycorrhizal colonization percentage (M%) of black and white poplars was higher in mycorrhizal plants than non-mycorrhizal ones (Figs 1 and 2), implying that mycorrhizal inoculation could increase the percentage of natural mycorrhization (Turnau 1998). In both poplars,

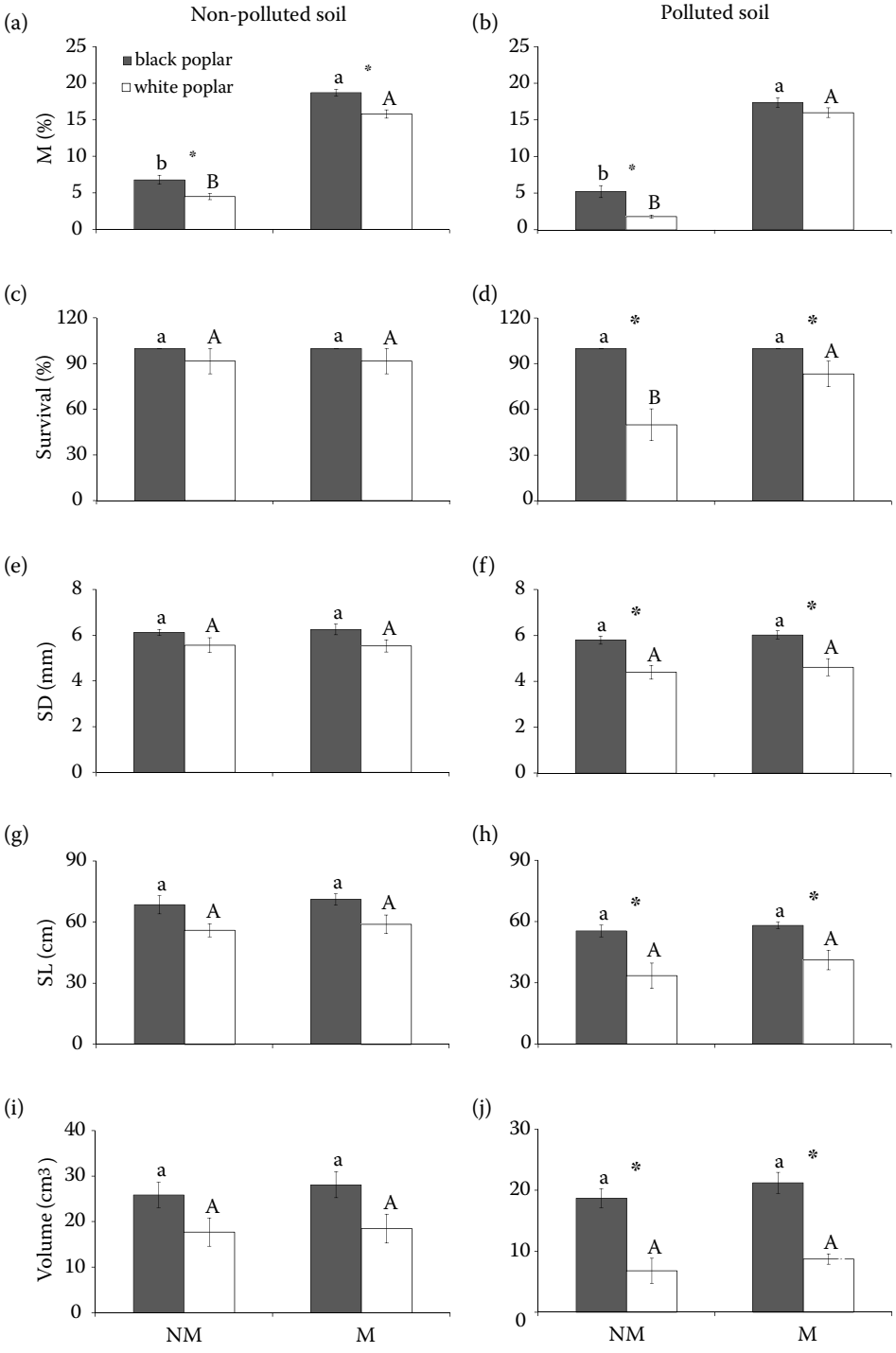

Fig. 1. Plant parameters of black and white poplars on non-polluted and polluted soils (1,000 mg Pb·kg^{-1}) in July. Plants were either not inoculated (NM) or inoculated with arbuscular mycorrhizal fungal community (M)

bars indicate standard errors, different small letters indicate a significant difference between M and NM treatments in black poplar, different capital letters indicate a significant difference between M and NM treatments in white poplar ($P < 0.05$), *significant differences in M or NM plants between black and white poplars at $P < 0.05$
SD – stem basal diameter, SL – stem length

M% of plants increased from July to October (Figs 1 and 2). The mycorrhizal colonization rate, in this study, for both poplars is in accordance with earlier data on poplars (Lingua et al. 2008; Cicatelli et al. 2010). As shown in Figs 1 and 2, M% of black poplar plants was significantly greater than M% of white poplar plants. In accordance with our results, the study of Lingua et al. (2008) also demonstrated that M% of black poplar plants on non-polluted and polluted soils (Zn) was higher than M% of white poplar plants.

One of the main factors for evaluation of the plant performance in the soil polluted with heavy metals is plant survival (Tanvir, Siddiqui 2010). In this study, all plants of black poplar on non-polluted and polluted soils survived up to 100%, but

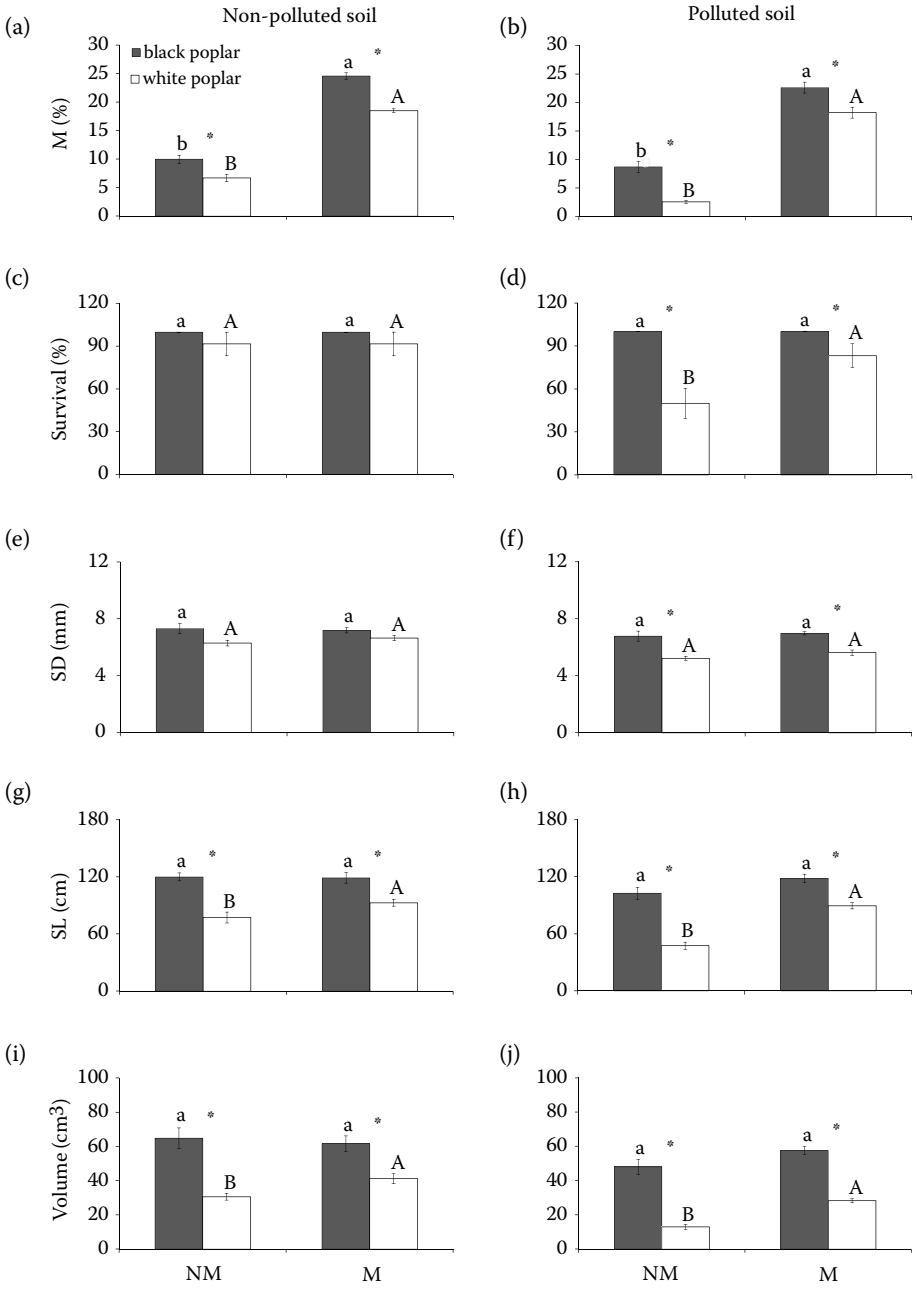

Fig. 2. Plant parameters of black and white poplars on non-polluted and polluted soils (1,000 mg Pb·kg^{-1}) in October. Plants were either not inoculated (NM) or inoculated with arbuscular mycorrhizal fungal community (M)

bars indicate standard errors, different small letters indicate a significant difference between M and NM treatments in black poplar, different capital letters indicate a significant difference between M and NM treatments in white poplar ($P < 0.05$), *significant differences in M or NM plants between black and white poplars at $P < 0.05$
SD – stem basal diameter, SL – stem length

in white poplar, survival rate ranged from 50 to 100%. In both poplars, survival rate was the same at both times (Figs 1 and 2). Non-mycorrhizal plants of white poplar grown in polluted soil (1000 mg $Pb \cdot kg^{-1}$) had the lowest survival (Figs 1 and 2). In fact, on polluted soil, in white poplar, mycorrhizal colonization enhanced the plant survival percentage, but the same trend was not observed in black poplar. On polluted soil, the survival of black poplar mycorrhizal and non-mycorrhizal plants was significantly higher than that of white poplar. We show here that various poplar clones and species have different survival on soils polluted with heavy metals, probably due to the broad genetic diversity of poplars (Aravanopoulos et al. 1999).

In both poplars, stem growth (SL, SD) and volume production of plants increased from July to October (Figs 1 and 2). In July, mycorrhizal inoculation with AM fungal community had no significant effect on stem growth (SL, SD) and volume production of black and white poplar plants grown on non-polluted and polluted soils (Fig. 1). However, in October, mycorrhizal plants of white poplar had greater SL and volume production than non-mycorrhizal plants. This trend was found in both non-polluted and polluted soils. In contrast, mycorrhizal and non-mycorrhizal plants of black poplar did not show any differences in growth and volume production (Fig. 2). It is reported that mycorrhizal inoculation could improve survival and growth of plants under heavy metal stress (Ouahmane et al. 2007; Bissonnette et al. 2010). However, the effect of AMF on plant parameters depends on many factors including plant and fungus species, growth condition of plant, plant age, soil properties, type and concentration of heavy metals of soil and etc. (Arriagada et al. 2005; Mrnka et al. 2012). For example, Lingua et al. (2008) reported that AMF inoculation of white poplar improved plant growth on Zn polluted soil, however this positive influence was not observed in black poplar. Also, Bissonnette et al. (2010) stated that inoculation with the fungus *G. intraradices* increased the biomass of *Salix viminalis* Linnaeus and *Populus* × *generosa* Henry in a soil polluted with Cd, Pb, Cu and Zn, however *P.* × *generosa* produced significantly more biomass than *S. viminalis*.

The comparison of growth and volume production of two poplars in July and October showed that the mycorrhizal and non-mycorrhizal plants of black poplar had higher growth and volume production than those of white poplar plants, especially on polluted soil (Figs 1 and 2). Our results are in agreement with growth responses of poplars on soils polluted with heavy metals as reported by Gu et al. (2007), Borghi et al. (2008) and Lingua et al. (2008).

CONCLUSION

The results of the present study indicated that while mycorrhizal inoculation improved survival, growth and volume production of white poplar plants on both soils in October, especially on Pb-polluted soil, mycorrhizal and non-mycorrhizal plants of black poplar did not show any significant differences on polluted and non-polluted soils. This suggests that in white poplar, mycorrhizal inoculation could improve the efficiency of plants, especially on Pb-polluted soils. On the other hand, all plants of black poplar had significantly higher survival, root mycorrhizal colonization, stem growth and volume production than white poplar plants on polluted and non-polluted soils. On polluted soil in October, volume production of white poplar was 62% less than that of black poplar. We conclude that the black poplar clone on Pb-polluted soil will perform better than the white poplar clone.

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