Influence of drought on allelopathic properties of *Pinus sylvestris* L.

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Received: 17.09.2019 | Accepted: 05.03.2020 | Published: 30.06.2020

**Abstract**

The objective of this study is to evaluate the influence of drought on the allelopathic activity of *Pinus sylvestris* L. plants at the M.M. Gryshko National Botanical Garden, National Academy of Sciences of Ukraine.

**Material and methods.** The choice of study objects was due to their habitat: plants of *P. sylvestris* grew in compliance with the necessary agricultural technology, including regular irrigation at site one (control) and in the conditions of drought at site two that models natural ecosystems (experiment). The allelopathic activity of leaves and soil was determined. In the model experiments, *Lepidium sativum 'Azhur', Triticum aestivum 'Poliska 90', Amaranthus caudatum 'Rothschwanz' were applied as the test plants.

**Results** of the current study revealed that the allelopathic potential of needles and rhizosphere soil of *P. sylvestris* depend not only on the species but also on the drought conditions. The inhibitory effect was stronger against root elongation than shoot elongation of seedlings.

**Conclusion.** This study provides laboratory-based evidence of the allelopathic effect of *P. sylvestris* exudates under drought stress on model plants. Studies showed that the plants that were grown under conditions of lower soil moisture exhibit a more substantial inhibitory effect or slightly stimulate the growth of test objects in comparison with plants that had a better water supply. This testifies to the relationship between soil moisture and seasonal allelopathic activity of the plants.

**Keywords:** *Pinus sylvestris*, drought, allelopathic activity

**Introduction**

*Pinus sylvestris* L. forests are sensitive to drought-related dieback. In a review of global forest mortality, *P. sylvestris* forests accounted for 40% (10 out of 25 cases) of all European die-off events (Allen et al., 2010). This situation may get worse and seriously impede forest regeneration in the future as climate change simulations predict increasing temperatures and decreasing local summer precipitation even in moderate scenarios (Intergovernmental Panel on Climate Change, 2014).

One of the significant response systems to prolonged drought pressure is that plants can change their growth and developmental phenotype to deal with an arid environment through long-term ecological adaptation, which is more linked with allelopathic regulation (Zuo et al., 2010). Allelopathy may be effectively employed for improving resistance against abiotic stresses. Production of allelochemicals serves as a tool for plant survival. They help to avoid, tolerate, and mitigate catastrophes in an efficient way (Farooq et al., 2013). In this way, allelochemicals significantly impart resistance against
environmental stresses and consequently make plants able to grow better. Environmental stresses trigger specific and non-specific stress-reaction regulated by a feedback mechanism (Pedrol et al., 2006). These reactions serve as an indicator of stress in plants and induce internal metabolic machinery through signaling and signal transduction processes. In this way, plants use secondary metabolites as messengers under suboptimal conditions to trigger the defense reactions (Isah, 2019).

The role of allelopathy in overcoming arid conditions, in conjunction with their allelopathic behavior, is still poorly understood. Some studies (Zhou, 1999; Kong et al., 2002) show that the synthesis of allelochemicals is more intensive in the adverse or harsh environment when water is limited. Plants often use allelochemicals to increase their competitive ability and, therefore, survival rates. It was demonstrated that water deficit condition is the driving force for metabolome shift in plants growing in arid or semi-arid soil conditions. Selective induction of a few metabolites and keeping a significant part of the metabolome unaffected by water deficit conditions may be a key strategy for water stress management in the desert plants. It is also suggested that homeostasis of natural compounds at the endogenous levels plays a crucial role in these species' ability to withstand periods of water stress (Friedjung et al., 2013).

It was shown on a wide range of ecosystem types that allelochemicals affect forest tree growth and development (Van Rooyen, 2004). P. sylvestris has the largest geographical distribution among pine species and is one of the most widespread conifers on Earth, distributed from the Mediterranean to the Arctic (Matias et al., 2012). Allelopathy is one of the factors, which cause the issues with the regeneration of the coniferous plants. A dense ericaceous understory that develops after forest harvesting or fire has high allelopathic activity (Mallik, 2003). In the coniferous forests that consist mainly of P. sylvestris, the dominant species of the understory at the clearcuts are mostly ericaceous. They produce and secrete a range of phenolic compounds. Most of them inhibit germination of the conifer seed germination (mainly primary root growth) and ectomycorrhizal growth (Šežiene, 2017).

Although numerous studies have been focusing on P. sylvestris allelopathy mechanisms, the allelopathic responses of it under drought are poorly understood. Therefore, the objective of this study was to assess the allelopathic activity of the needle extracts and root exudates of P. sylvestris under drought stress. The results of this study provide a better understanding of the allelopathic responses of P. sylvestris under drought, expected to occur concurrently in a changing climate. It can, therefore, be assumed that results obtained regarding the changes in the allelopathic potential of dominant species P. sylvestris may be important. They can improve our understanding of the impact of allelopathy on reforestation and management of forest ecosystems under changing climatic conditions and also understanding how to use allelopathy as a tool for forest management in general.

Material and methods

The choice of study objects was due to their habitat: 60-year-old P. sylvestris plants, which grew in compliance with the necessary agricultural technology including regular irrigation at site one (control) and in the conditions of drought at site two that models natural ecosystems (experiment). The needles from branches in lower crown and soil samples were collected for 10 randomly chosen trees at each sampling site. The canopy at the site one was formed by P. sylvestris with about 50 trees (Fig. 1). The height of the trees is about 25 m, and the diameter at breast height (DBH) is 0.39 m. The shrub layer is absent. The first layer at the test site two was formed by about 30 trees of P. sylvestris (Fig. 2). The height of the trees is 15 m, and DBH is 0.29. The undergrowth is represented here mostly by Quercus robur L. with the height from 1 to 2.5 m.

Germination tests were carried out at the M.M. Gryshko National Botanical Garden. Allelopathic activity of aqueous extracts of P. sylvestris was estimated using seed germination bio-assay (Baležentienė & Sirgedaitė-Šėžienė, 2010). Freshly collected mature (one-year) needles were chopped into 0.5 cm long pieces before the extraction of allelochemicals. Fifty grams of each piece were immersed in a 15 × 20 × 5 cm plastic tray containing 250 ml of distilled water. Containers were closed with glass plates and kept at
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5°C in an incubator. After 12 h, the aqueous extracts were filtered through Whatman no. 1 filter paper, diluted and used for germination assays. Fifteen sterilized seeds were placed on filter paper in a closed Petri dish (ø 9 cm). After that, the Petri dishes were incubated in the dark at 27°C for 48 h. Five replications were applied per each treatment. The distilled water served as a control. The values were expressed as relatives (%) to the control.

Allelopathic effects of the soil were assessed *in vivo*; bio-assay was performed following Wu et al. (2001). *Triticum aestivum* L. 'Poliska 90' was germinated in Petri dishes (ø 6 cm) containing 4 g of soil taken from the field. The soil was collected from the upper 1-cm layer within a 10-cm radius of the plant base. About 4 ml of distilled water was added, and 10 sterilized seeds were placed on the wet soil. Closed Petri dishes were incubated in the dark at 27°C for 48 h. As a control, the soil from the site without plants was applied. Each treatment was tested in five replicates. The values were expressed as relatives (%) to the control.

In the model experiments, as the test-plants were used *Lepidium sativum* L. 'Azhur', *Triticum aestivum* 'Poliska 90' and *Amaranthus caudatum* L. 'Rothschwanz'.

The results were expressed as means with the least significant difference (LSD). The significance of differences compared to the control groups was determined using the t-test.

**Results and discussion**

Changing environmental factors and allelopathic relations are commonly present at the same time in many plant populations

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**Figure 1.** *Pinus sylvestris* plants at the site 1.

**Figure 2.** *Pinus sylvestris* plants at the site 2.
(Reigosa et al., 1999). So, the induction of secondary metabolites in plants as a response to the stress can be considered a ‘cost-saving’ mechanism, because such metabolites are induced as defense and also used as allelochemicals (Siemens et al., 2002; Hoballah et al., 2004). Recent works have shown empirical evidence of selective advantages that allelopathic activity provides to many plant species (Oliva et al., 2002; Florentine, 2003), especially under such stress conditions like drought (Kong et al., 2002).

Pinus sylvestris has an exceptionally high concentration of terpenes, resin acids, and phenolics in the needles, which generally inhibit the germination of seeds, but in low concentrations stimulated root and shoot elongation of certain turfgrass varieties (Bulut & Demir, 2007). It was found that allelopathic inhibitory effects of P. sylvestris aqueous extracts depend on temperature impact. In particular, inhibitive properties of extracts increased with increasing temperature and resulted in decreased seed germination, hypocotyl height, and root length (Sirgedaite-Šežiene et al., 2019). Aqueous extracts from fresh needles of P. sylvestris inhibited the root and shoot elongation of test seedlings (Fig. 3). The inhibitory effect was stronger against root elongation than shoot elongation of seedlings. Needle extracts reduced the growth of seedlings in up to 9.4 times.

Pinus sylvestris is an important source of biogenic volatile organic compounds, which emission rate and profile affect air quality, climate forcing, plant stress tolerance, and thus the growing conditions of forests (Kivimäenpää et al., 2018). The reaction of P. sylvestris to various stress factors (low temperatures, industrial pollution, and action of fungi) showed changes in the content and composition of secondary metabolites (phenols, resins, essential oils). The components of the essential oil in the needles reacted to stress most quickly – their synthesis is removed with the formation of volatile substances, especially α-pinene. This can be an optimal indicator of early diagnosis of the state of P. sylvestris. Changes that occur in the secondary metabolite in P. sylvestris needles under the influence of stress factors can be considered as a non-specific reaction to stress (Fuksman, 2002).

In addition, terpene emission from plant species predicted to increase substantially due to a warmer climate and dense vegetation communities (Peñuelas & Llusià, 1999; Peñuelas & Staudt, 2010). This supports the need for further research on the role played by these volatile organic compounds in ecosystem functioning. In this context, it is of prime interest to improve our knowledge about the allelopathic potentialities of P. sylvestris volatile compounds.

The volatile compounds of pine needles differ from the braking effect on the development of all test objects. The volatiles from the needles of P. sylvestris significantly decreased the seedling growth of two test plants (Fig. 4). The highest inhibitory effects of volatiles were on the roots of A. caudatum and T. aestivum (12.08

**Figure 3.** Effect of aqueous extracts from fresh needles of *Pinus sylvestris* on seedling growth. The error bars represent LSD.
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1.3–18.1 times higher in spring, 1.4–19.5 times higher in summer, and 1.4–26.7 times higher in autumn comparing to the areas where the test plants grow.

In *P. sylvestris* structural acclimation to drought involving increased biomass allocation in the roots, in comparison with shoots (Seidel & Menzel, 2016). When soil from the site where regular irrigation was conducted, results confirmed that seedling growth was very poor in soil under drought (Figs. 5–7); that may be due to the presence of allelochemicals as well as the changes in soil nutrient availability. The mechanisms involved in nutrient dynamics changes in the soil in relation to allelochemicals were described (Jabran et al., 2013). Typically, allelochemicals are considered to have a substantial role in nutrient availability and nutrient cycling in the ecosystem (Appel, 1993). In our research, the reduction in seedling growth depended on the concentration of allelochemicals and season of the year.

Germination and seedling development are the main life stages usually affected by allelochemicals. Frequent allelopathic influence often inhibits (Fernandez et al., 2013; Santonja et al., 2019) and results in a delay of seed germination (Fernandez et al., 2013; Hashoum et al., 2017). It also inhibits the seedling growth (Gavinet et al., 2019) by altering physiological processes (e.g., photosynthesis, nutrient uptake, cell division, or elongation (Inderjit & Duke, 2003)). The direct bioassay of the rhizospheric soil under *P. sylvestris* showed that the growth of the roots of *L. sativum* was 1.5 times slower in spring and 1.5 times slower in summer and autumn compared to the experimental plants (Fig. 6). Water extracts of soil under *P. sylvestris* from the experimental area suppressed root growth in 1.3–1.6 times (depending on the concentration) relatively to the control plants.
Figure 5. Effect of aqueous extracts from the soil of *Pinus sylvestris* on seedling growth of *Lepidium sativum*. The error bars represent LSD.

Figure 6. Effect of direct bio testing of rhizospheric soil of *Pinus sylvestris* on seedling growth of *Lepidium sativum*. The error bars represent LSD.

Figure 7. Effect of volatiles from the soil of *Pinus sylvestris* on seedling growth. The error bars represent LSD.
The volatiles are also the potential inhibitors of seedling growth and germination (Eom et al., 2006). It was proved that some volatiles could be dissolved and absorbed by the soil (Reigosa et al., 1999). This is especially important for woody plants, which grow and accumulate allelochemicals in the soil for a long period of time. The estimation of the influence of volatile soil substances on the growth of the test cultures showed that the linear dimensions of the coleoptiles and the roots of T. aestivum were 1.1–1.4 times smaller, and the roots of A. caudatum – 1.1–1.6 times smaller, depending on the season (Fig. 7).

Conclusions

The results of the current study revealed that the allelopathic potential of needles and soil from P. sylvestris depended on not only from species itself but also on drought conditions. Studies have shown that plants grown under conditions of lower soil moisture exhibited a stronger inhibitory effect or less stimulated the growth of test objects in comparison with pine plants that had a better water supply. This testifies to the relationship between soil moisture and allelopathic activity of the plants. Detailed allelochemical insight of plants under water stress conditions will be a further endeavor.

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Influence of drought on allelopathic properties of *Pinus sylvestris*

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Мета – з’ясувати вплив посухи на алелопатичну активність хвої та ризосферного ґрунту з-під рослин *Pinus sylvestris* L. в умовах Національного ботанічного саду імені М.М. Гришка НАН України.

Матеріал та методи. Вивчали 60-річні рослини *P. sylvestris*, які росли в умовах 30% (дослід) і 60% (контроль) вологості ґрунту. Зокрема, визначали алелопатичну активність екстрактів хвої та ґрунту. Тестовими культурами слугували крес-салат (*Lepidium sativum* ‘Azhur’), пшениця (*Triticum aestivum* ‘Poliska 90’) та амарант (*Amaranthus caudatum* ‘Rothschwanz’).

Результати. Виявлено, що леткі речовини хвої інгібували ріст усіх тест-об’єктів. Крім того, леткі речовини хвої рослин з дослідної ділянки пригнічували ріст тестових рослин більше, ніж з контрольної ділянки. Аналіз алелопатичної активності ризосферного ґрунту з дослідної ділянки показав, що приріст коренів *L. sativum* був у 1,5 рази меншим в усі пори року. Водорозчинні речовини ґрунту з дослідної ділянки пригнічували ріст коренів у 1,3–1,6 разів сильніше, ніж з контрольної ділянки. Леткі речовини ґрунту інгібували ріст колеоптилів та коренів *T. aestivum* у 1,1–1,4 рази, а коренів *A. caudatum* – у 1,1–1,6 разів залежно від пори року. Інгібуючий ефект був сильніший щодо росту коренів, аніж щодо колеоптилів.

Висновок. Встановлено, що рослини, які вирощувалися в умовах меншої вологості ґрунту проявляли сильніший інгібуючий вплив і більше стимулювали ріст тест-об’єктів у порівнянні з рослинами, ґрунт яких характеризувався більшим вмістом води. Це свідчить про залежність між вологістю ґрунту та сезонною алелопатичною активністю досліджених рослин.

Ключові слова: *Pinus sylvestris*, посуха, алелопатична активність