Evaluating the effect of culture filtrate of *Trichoderma harzianum* on the seed germination and seedling growth of *Pinus sylvestris*

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Abstract. Researchers are increasingly drawn to exploring additional potential advantages of Trichoderma spp. as the use of these microorganisms in agricultural and forestry applications, known for their notable biological activities, continues to gain popularity. In this work the isolate of *Trichoderma harzianum* was tested to evaluate its efficacy on the germinating of the common pine seeds and additionally, how growing the fungi on different mediums can affect its biological activities. Findings in this work proves that Trichoderma can grow differently in various media and have diverse sporophytes that differ morphologically. Moreover, using the culture filtrate of Trichoderma harzianum can enhance the germination process of the seeds and the growth of the seedlings.

1 Introduction

Trichoderma, a fungal genus found ubiquitously in soils, stands out as the most abundant and cultivable fungi. Within this genus, numerous species can be classified as opportunistic symbionts of plants, displaying avirulent characteristics [1]. Recognized since 1887 for their antagonistic qualities, Trichoderma species have been extensively employed as biological control agents. Interestingly, several Trichoderma species engage in direct physical interactions with plants, leading to advantageous outcomes such as enhanced vegetative and root growth, improved nutrient availability, increased yield, and heightened resistance against diseases [2,3].

The utilization of Trichoderma spp. presents itself as a viable approach to address challenges in agriculture and forestry. Employing biological control through Trichoderma spp. proves effective in curtailing the proliferation of plant-damaging microorganisms and modulating plant growth rates.[4] The role of Trichoderma spp. in inhibiting the growth of harmful microorganisms and promoting plant growth has been demonstrated through the secretion of secondary metabolites [5]. Moreover, the successful interaction between Trichoderma spp. and plants effectively regulates root architecture, resulting in increased length of lateral and primary roots. This, in turn, enhances the plant's ability to uptake

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nutrients efficiently. In addition, Trichoderma spp. are recognized for their ability to induce plant defense responses, stimulate plant growth through the production of growth hormones, antibiosis compounds, and cell wall degrading enzymes, and trigger broad spectrum systemic resistance responses (ISR) in leaves[6].

By coating seeds with Trichoderma spp., the need for chemical seed treatments can be eliminated. This allows for the establishment of seed bio-priming, where beneficial microorganisms become readily available to crop roots. This process enables Trichoderma spp. to colonize the rhizosphere during the crucial "early germination" stage, promoting early, robust development, improving nutrient absorption, and enhancing tolerance to various stresses[7,8].

There is currently limited knowledge regarding the early interactions between seeds and Trichoderma spp. However, these interactions hold significance for two main reasons:

(1) they can offer valuable insights into the long-term performance of plants, and (2) if properly characterized and quantified, seed-Trichoderma spp. interactions can serve as effective and rapid systems to investigate the mechanisms and physiological processes involved in plant-Trichoderma spp. interactions [9]. Furthermore, since these fungi are commonly used as seed treatments, they have the potential to enhance plant stands and promote lasting enhancements in plant quality. As a result, seed treatments have the capacity to bring about both short-term and long-term improvements in seed quality and subsequent plant performance [10].

Currently there are significant number of studies investigating the importance of several species of Trichoderma in agriculture and forestry, and in this work the effect of *Trichoderma harzianum* on the germination of common pine seeds and on the growth of the vegetative parts was investigated.

2 Methods

2.1 Plant material

Seeds of common pine *Pinus sylvestris* were received from the forest seed station Pushkino, Moscow region. Collected in 2021.

2.2 Fungi Isolate

The used strain *Trichoderma harzianum* Th5 was obtained from Bioresource center All-Russian Collection of Industrial Microorganisms (BRC VKPM) National Research Center “Kurchatov Institute”.

2.3 Medium preparation

Three different media were used in this experiment, (i) Chapeka, (ii) Macro Soil medium (MS) and (iii) Woody Plants medium (WPM) in volume 500mL to grow *Trichoderma harzianum.*

12.5g of each MS and WPM media in dry form (concentration 25 g.L) was dissolved in 200 mL of distilled water in addition to 15 g of Saccharose 25 g.L and then the final volume was brought to brought to 500 mL.

2.4 Preparing culture filtrate

Trichoderma harzianum was incubated in liquid media of MS and WPM for 10 days where it grew and formed sporophytes, after incubation the culture was filtrated through a double layer of filter paper and this culture filtrate is considered as the concentration 100%. This concentration was used to prepare dilutions.

2-fold (50%) and 4-fold (25%) dilutions were prepared using distilled water and was kept for further experiments.

2.5 Planting seeds

For each concentration of culture filtrates (MS and WPM) plates (n = 3) were prepared, distilled water was used as control. Double layer of filter paper was placed in glass Petri dishes and soaked with each concentration of the culture filtrates, then 20 seeds of pine were placed and the plates were sealed and kept inside a light room to germinate.

2.6 Germination and growth monitoring

After 5 days of incubation the number of the germinated seeds were counted and used to calculate the percentage, then observation was repeated each 5 days twice and metric parameters were measured (the length of the roots and the vegetative part) and used for statistical analysis.

2.7 Statistical analysis

The variables were expressed as the average percentage ± standard deviation (SD). Statistically significant differences were determined using ordinary two-way ANOVA with Dunnett's multiple comparisons test. Differences were considered statistically significant at $P<0.05$.

3 Results

After incubating *Trichoderma harzianum* in MS and WPM media for 10 days the fungi started to grow and form sporophytes, the morphology of the sporophytes differed drastically between the two used media.

In the MS media the sporophytes had a spherical shape, with white to grey color and few numbers while in the WPM medium they were more round with yellow coloration and higher numbers (figure 1). This clearly implies the direct effect of the used medium on the growth of the fungi and it demands further biochemical analysis in order to understand how the structure of the medium affect the synthesis of fungi hyphae and pigmentation.

The effect of the Trichoderma Spp. on the germination of the seeds was addressed before by large amounts of studies on both crop and forest plants. For example, in the work of kthiri et al. [11] coating the seeds of durum wheat with different Trichoderma strains enhanced the growth of the plant and its resistance to some soil pathogens such as Fusarium species. Additionally in the study od Hohmann et al. [12] treating the seedlings of *Pinus radiata* with Trichoderma improved the seedlings performance and Trichoderma populations across treatments significantly decreased in the rhizosphere between 2 and 3 m corresponding to the time of seedling transfer from the glasshouse to the open area.

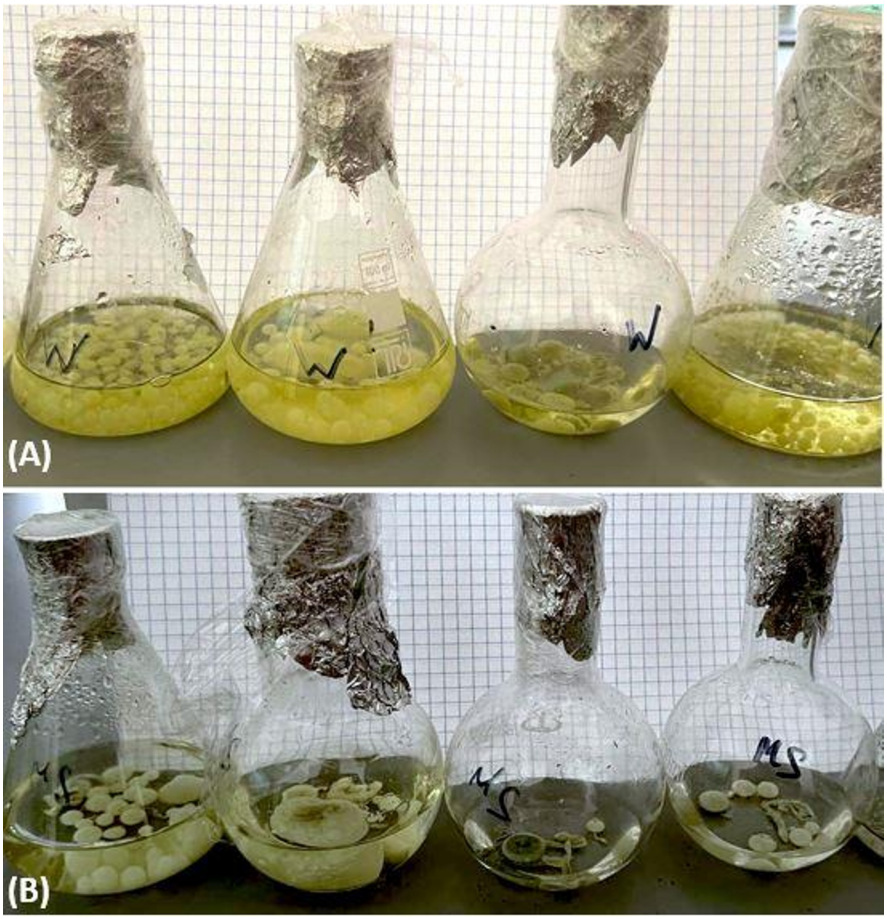

Fig. 1. Shows the different morphology of the sporophytes of *Trichoderma harzianum* in (A) WPM medium and (B) MS medium.

Similarly in our work the results of comparing how culture filtrates of different concentrations effect the germination, indicates that the WPM was more efficient than MS during the first 10 days, and specifically the WPM 50% gave the best results with around 70% of germinated seeds followed by WPM 25% with around 60% in comparison to distilled water (only about 52%). MS filtrate provided the lowest values in this context (Figure 2).

These results can indicate that since the Trichoderma is opportunistic symbionts of plants and especially forest plants, it shows a preference for the WPM and enhance the germination process in a higher rate when compared to only water or MS medium.

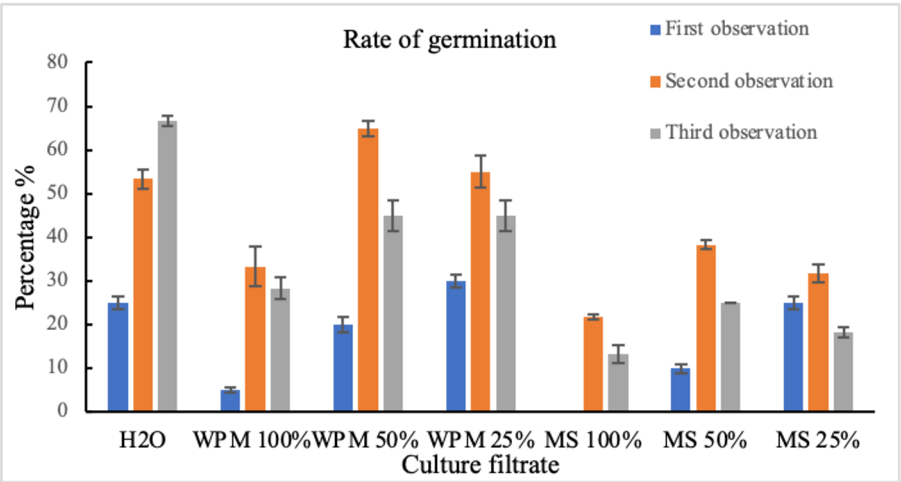

Fig. 2. Demonstrates the percentage of germinated pine seeds treated with different culture filtrate. The values represent the percentage with standard deviation.

It is worth mentioning that after the 10th day the seedlings treated with filtrates started to die and this is mainly because of fungal over growth and contamination with black mold, and the growth in MS was more significant than other dishes (figure 3).

It was mentioned in a previous study by Li Destri Nicosia et al. [13] that some strains of Trichoderma can have a negative effect and cause the dieback of pine seedlings and specifically *Trichoderma viride*.

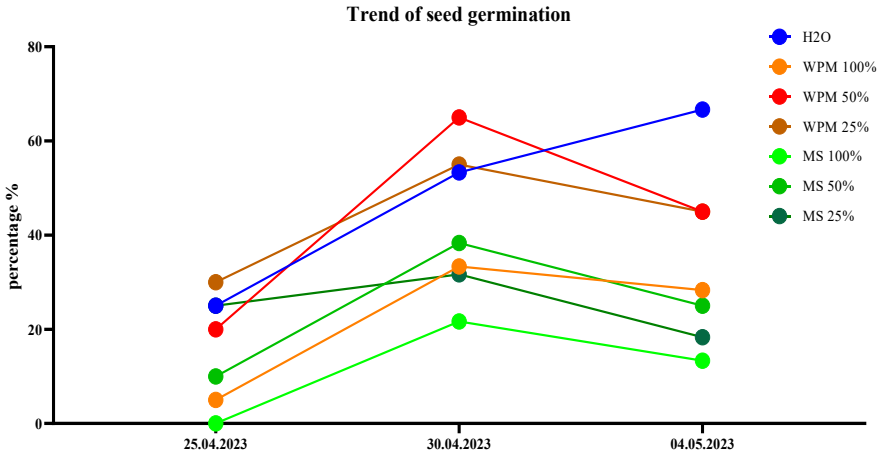

Fig. 3. Represents the germination trend during the time of experiment, and how fungal overgrowth effects the germination

Finally, when evaluating the growth of the roots and vegetative part of the seedlings water had the best effect on the length followed by WPM 25% and then WPM 50%.

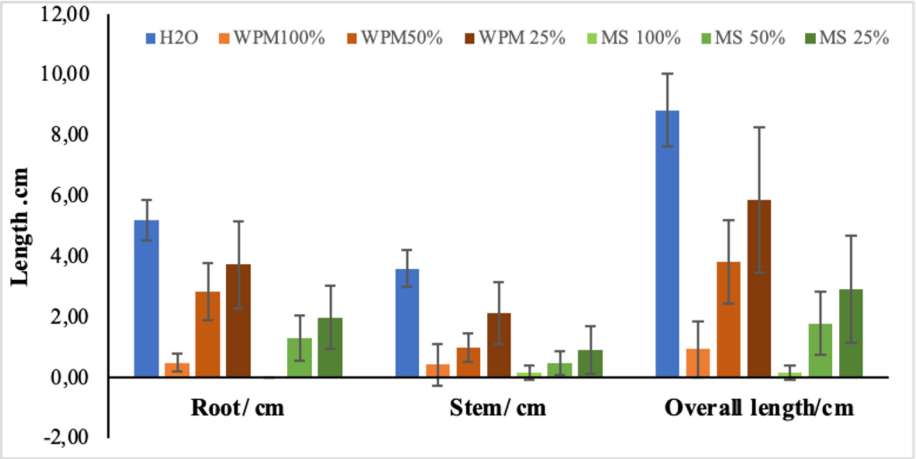

Fig. 4. Demonstrates the metric measurements of the roots and vegetative parts, and how culture filtrates affect the growth.

4 Conclusion

To sum up the WPM medium can provide a better substance for growing *Trichoderma harzianum* when compared to MS and the filtrate of the culture can induce high germination rate at the concentration 50% while it induced better growth of both roots and leaves at the concentration of 25%. Moreover, the culture filtration should be done through additional types of filters in order to avoid fungal overgrowth and subsequently the death of the seedlings, and even the studies species proved useful and effective but not all strains of Trichoderma are suitable for application. Finally, further biochemical investigations concerning how the chemical composition of the medium affect the fungi is required in order to provide a better understanding.

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