Effect of biochar and beneficial microorganisms on white root rot disease on Japanese apricot plants

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Research Article

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Abstract

Biochar as a soil conditioner is known for affecting several soil and plant properties as well as nutritional status. It can also aid the suppression of soil-borne pathogens. *Rosellinia necatrix* is a soil-borne microorganism that causes white root rot disease in a large number of plant species, especially fruit trees. The fungus, which occurs worldwide, is very aggressive and difficult to control. Arbuscular mycorrhizal fungi (AMF) and Trichoderma are beneficial microorganisms (BM) known to aid in soil-borne disease suppression. Biochar has an active effect on the symbiotic relationship between plants and BM. Thus, the objective of this work was to investigate the effect of biochar and BM on *Rosellinia necatrix* suppression on Japanese apricot. The research was held in 2 phases. In phase 1, performed *in vitro*, we assessed *R. necatrix* and *T. atroviridae* growth in biochar-enriched medium. In phase 2, we inoculated Japanese apricot plants with *R. necatrix* and assessed disease severity. We discovered that *R. necatrix* was suppressed by *T. atroviridae* more efficiently than the control treatment when rice husk biochar was applied at a 0.5% concentration *in vitro*. We also learned that when rice husk biochar was amended, the effects of *R. necatrix* on Japanese apricot plants were less severe when compared to the control treatments, with disease progress being slower. We concluded that Rice husk biochar amendment successfully suppressed white root rot disease progression *in vitro* and *in vivo*, while bamboo biochar slowed disease progression *in vivo*. Mycorrhiza did not suppress *R. necatrix* growth.

Article Highlights

- Rice husk biochar amendment was an efficient method in controlling white root rot disease *in vivo*.
- Low concentrations of rice husk biochar (0.5%) favored *T. atroviridae* growth over *R. necatrix* *in vitro*.
- Biochar slowed white root rot disease progression.

1 Introduction

It is estimated that the world population will increase by more than 26% in 2050 (UN 2023) and rising incomes in developing countries will elevate the global demand for food (FAO 2023), thus elevating the demand for higher crop yields. However, the intensive use of use land for agriculture is a challenge because it can lead to higher Greenhouse Gas emissions, soil degradation and waterbed contamination (Mengel 1993). One of the most important tools that can be used to mitigate these problems and to move towards a more sustainable food production future is biochar.

Biochar is a carbon-rich material, produced through the thermochemical conversion of a biomass source, in an environment of low oxygen availability. The resulting material is known for increasing soil organic matter, suppressing soil pathogens, sequestering carbon, mitigating NO₂ emissions, enhancing soil microbiome, among other benefits (Bailey et al. 2011; Graber et al. 2014; Jaiswal et al. 2018b). Biochar amendment also significantly enhances soil chemistry and enzyme activity, properties that are extremely correlated with soil bacterial community (Gao et al. 2019).
Biochar can control soil-borne pathogens (Graber et al. 2014; Jaiswal et al. 2018) with some of the proposed mechanisms including immobilization and deactivation of pathogenic enzymes and toxic metabolites, induced plant resistance and enrichment of soil bacterial abundance (Jaiswal et al. 2018a; Jaiswal et al. 2020). However, this phenomenon has high variability, most likely due to biochar property changes caused by pyrolysis temperature, quantity applied, particle size and material, with some results showing pathogen growth, instead of suppression, or no changes at all with biochar amendment (Jaiswal et al. 2014), thus indicating that studies with specific pathosystems should be conducted in order to gain more insights on biochar disease suppression mechanisms. Biochar dosage has a U-shaped response curve with fungus growth, with intermediate doses resulting in the best disease suppression. High doses stimulate disease, which is known as the hormesis effect. The host-pathogen system has a specific relationship with the type and dosage of biochar (Zwart and Kim 2012; Jaiswal et al. 2014; Frenkel et al. 2017).

Using BM to promote plant growth and suppress plant disease is seen as a viable alternative to the use of chemical fertilizers and pesticides in agricultural fields. *Trichoderma spp.* are BMs commonly found in soil and wood as inhabitants, symbionts of plants and mycoparasites (Schmoll and Schuster 2010). Some species of this genus are highly versatile and are currently used as active components in biofertilizers, biopesticides, growth enhancers, and natural resistance stimulants (Woo et al. 2014; López-Bucio et al. 2015; Coelho et al. 2021; Rao et al. 2022). Combining *Trichoderma spp.* with biochar can effectively control soilborne pathogenic fungi (de Araujo et al. 2019). Biochar also enhances the survival and growth of *Trichoderma spp.* populations, leading to a significant reduction in soilborne pathogens (Woo et al. 2014; Rao et al. 2022).

AMF are BM that can improve crop yield (Bakry et al. 2014; Jabborova et al. 2021), increase P and N uptake (Leigh et al. 2009; Li and Cai 2021; Qin et al. 2022) and have known biocontrol properties, including against *R. necatrix* (Elmer and Pignatello 2011; Cruz et al. 2014; Cruz 2015; Cruz-Paredes et al. 2019; Holland et al. 2019). It is also widely known that biochar can stimulate mycorrhizal growth (Holland et al. 2019; Jabborova et al. 2021; Qin et al. 2022).

According to Wang et al. (2020) the application of biochar at a 2% concentration can mitigate negative plant-soil feedback by modifying the soil microbiome. This is largely due to the fact that biochar soil amendment enriches beneficial bacteria with antagonistic activity against pathogens, thus inhibiting their growth. Wu et al. (2020) found that the abundances of potentially beneficial *Pseudomonas spp.*, *Burkholderia spp.*, and *Bacillus spp.* increased under rice husk biochar amendment at a concentration of 3% within three weeks.

*Rosellinia necatrix*, an ascomycete soil-inhabiting fungus, causes white root rot disease in a large number of plant species, especially fruit trees (Pliego et al. 2012). The fungus, which occurs worldwide, is very aggressive and can kill infected trees, and the symptoms on the shoots usually only appear when the roots are already severely infected (Perez-Jimenez 2006). Studies carried out with sterilized and non-sterilized soils show that *R. necatrix* has a reduced colonization ability in the presence of other soil
microorganisms. (Mantell and Wheeler 1973), showing the importance of maintaining a healthy soil microbiome to prevent soil-borne diseases.

In this study, we examined the effects of the joint use of biochar and AMF on white root rot disease \textit{in vivo} and the effect of biochar on the suppression of \textit{R. necatrix} through \textit{T. atroviridae in vitro}. We hypothesize that biochar will enhance the efficiency of BMs on white root rot disease control.

2 Materials and methods

2.1 Experiment 1: Direct effect of biochar concentrations on \textit{R. necatrix} and \textit{F. oxysporum} suppression by \textit{T. atroviridae}

\textit{Collection and selection of disease causing and biocontrol isolates:} \textit{T. atroviridae} (NBRC 8436) and \textit{R. necatrix} (NBRC 5954) were acquired from National Biological Resource Center, NITE (NBRC) in Japan. \textit{F. oxysporum} was isolated and identified previously in our lab, and its sequence deposited in DNA Data Bank of Japan. The fungi were then multiplied and re-cultured in PDA medium. For the biocontrol, 3 different species of \textit{Trichoderma} were tested, but \textit{T. atroviridae} was the only one that showed efficient biocontrol and thus selected to carry on with the experiment.

\textit{Biochar characteristics:} Commercial bamboo biochar (Nakamura Mfg. Co., Ltd, Kagoshima 100) was characterized as follows: Total N 1.5\%, total C 75\%, total P 1\%, total K 0.3 \%, pH 10. Commercial rice husk biochar (Green Bran Co.) was grinded to be more easily applied into the Petri dishes and characterized as follows: Total N 0.6\%, total C 43.5\%, total P 0.07\%, total K 0.05\%, pH 6.5.

\textit{Single culture method:} The three fungi were placed in the middle of Petri dishes (9 cm diameter) with PDA medium and amended with 0\%, 0.1\%, 0.25\%, 0.5\% and 1\% of both biochars with three repetitions for each treatment. 50\% PDA medium was used in all treatments except for the treatment containing 0\% biochar. After inoculation, plates were incubated at 25\textdegree{}C for 3 days. Then, hyphal growth was measured for 3 consecutive days.

\textit{Dual culture method:} Selected hyphae of the isolates of the pathogenic fungi and the biocontrol were placed in opposite positions on Petri dishes containing PDA and biochars at concentrations of 0\%, 0.1\%, 0.25\%, 0.5\% and 1\%, with three repetitions for each treatment. 50\% PDA medium was used in all treatments except for the treatment of 0\% biochar. The treatments were as follows: \textit{R. necatrix} + \textit{T. atroviridae} and \textit{F. oxysporum} + \textit{T. atroviridae}. The concentrations were based on previous works by de Araujo et al. (2019) and de Araujo et al. (2021), which showed that concentrations below 1\% had a direct effect on pathogen suppression while not affecting the tested biocontrol agent.

2.2 Experiment 2: \textit{In vivo} effect of biochar and AMF on white root rot severity
Japanese apricot pot experiment: Koshuko variety Japanese apricot was sown in trays during spring (April) and transplanted to 3.5 L pots in June. Biochars were applied at a concentration of 2% for its proven effects on soil microorganisms (Ishii and Kadoya 1994; Farrell et al. 2013; Song et al. 2014; Zhou et al. 2019; Jaiswal et al. 2020; Yan et al. 2021a; Dror et al. 2022), 5g of commercial mycorrhizal mixture containing mostly *Glomus* genus (Applied Natural Science Co., Bara Kinkonkin), and organic fertilizer was applied via fertirrigation containing 25, 5 and 50 ppm of N, P and K, respectively. In addition, pots were also fertilized with 2.625 g of MgSO₄. Plants were irrigated until field capacity every other day through an automatic sprinkler system. For the inoculated treatments, pots were inoculated 2 years after transplanting and measured. In all treatments, at harvest time, shoots and roots were cut and placed in paper bags for fresh mass and leaf N and P measurements (only shoots). Root samples were taken and placed in FAA (Formalin, acetic acid, and alcohol) and staining was conducted according to method previously described by Phillips and Hayman (1970).

Inoculation of *R. necatrix*: 400 g of wheat (*Triticum aestivum*) seed was soaked in 300 ml of distilled water in a glass flask. The flask containing sterile seed was autoclaved and then inoculated with 1 piece (9 by 9 cm) of a 7-day-old culture of an *R. necatrix* isolate. The flask was incubated for 15 days at 24°C in the dark, until *R. necatrix* grew uniformly, completely covering the wheat seed, grinded through a blender and then immediately inoculated directly in the plant pots.

*Treatments for pot experiment*: Control (c), control + mycorrhiza (c+gm), control + *R. necatrix* (c+rn), rice husk biochar + mycorrhiza (brh+gm), rice husk biochar + *R. necatrix* (brh+rn), rice husk biochar + mycorrhiza + *R. necatrix* (brh+gm+rn), bamboo biochar + mycorrhiza (bb+gm), bamboo biochar + *R. necatrix* (bb+rn), bamboo biochar + mycorrhiza + *R. necatrix* (bb+gm+rn).

*Fresh weight*: Fresh weight of shoots and roots was measured immediately after harvest using a scale.

*Leaf N and P*: Sulfuric digestion and colorimetry were performed, according to Eastin (1978). Plant leaves were separated from shoots then grinded into powder form, and 0.1g of sample was placed in test tubes. 1 mL of H₂SO₄ and 0.06g of sodium thiosulfate was poured in the test tubes and let sit for 12 hours. Samples were then heated for about 3 hours (until white smoke can be seen) at 150 – 200°C. After cooling, 500 µL of H₂O₂ was added and samples were heated for 30 minutes at 200°C. This process was repeated until the resulting liquid became transparent. 9 mL of distilled water was added to the test tubes, then the liquid was filtered and analyzed by colorimetry.

*White root rot severity assessment*: Inoculated plants were assessed for disease severity starting from 6 days after inoculation to 26, every 2 days. A score was given according to shoot symptoms (0 = no symptoms, 5 = dead plant).

*Statistical analysis*: Data was analyzed using a one-way ANOVA, followed by Tukey’s HSD test (P<0.05) to detect statistically significant differences among all treatments. All statistical analyses were performed using the software IBM SPSS Statistics, and graphs were elaborated using Microsoft Office Excel software, version 16.7.
3 Results and discussion

3.1 Experiment 1

*Single culture method:* all biochar treatments showed more hyphae growth than the control for the two fungi.

*Dual culture method:* the treatment containing *R. necatrix* + *T. atroviridae* with rice husk biochar at 0.5% showed significantly greater *T. atroviridae* hyphal growth than the control and less hyphal growth for *R. necatrix* than the control (figure 1). The remaining treatments showed no significative difference when compared to the control. Also, a thinning in *R. necatrix* hyphae was observed in the previously cited treatments (figure 2).

**Experiment 2**

*Shoot fresh weight:* The treatments c+gm, brh+gm, bb+gm showed higher shoot fresh weight when compared to the control. Treatments containing biochars showed slightly higher values than the mycorrhiza only amended treatment (c+gm).

*Root fresh weight:* Compared to the control (c), c+gm, brh+gm, bb+gm, had significantly higher values (table 1)

*Mycorrhizal colonization:* The treatment bb+gm had the same mycorrhizal colonization percentage as the control treatment amended with mycorrhiza (c+gm). The remaining treatments had significantly less colonization when compared to c+gm (figure 4).

*Leaf N and P:* Compared to the control treatment, only c+gm showed significantly higher leaf N percentage. The remaining treatments had lower leaf N percentages (table 1). For leaf P percentage, brh+gm and bb+gm treatments had higher results when compared to the control. The remaining treatments did not differ from the control (figure 4). Overall, the combination of biochars with mycorrhiza showed higher percentages of leaf P than c+gm.

*White root rot severity:* The control treatment plants died 14 days after inoculation. Brh+rn, brh+rn+gm, bb+rn and bb+rn+gm showed severity scores below 4 on the same day. Disease progression for these 4 treatments was also slower than the control treatment. Brh+rn plants had an average score below 4 even 26 days after inoculation (figure 3).

Our results suggest that low concentrations of biochar contribute to *R. necatrix* suppression by *T. atroviridae in vitro*, probably due to the fact that the biocontrol agent can take advantage of biochar-derived C more efficiently than the pathogen. Similar results were found by Jaiswal et al. (2014) where, in general, greenhouse waste and eucalyptus wood biochar addition at relatively lower concentrations enhanced cucumber growth performance and suppressed damping-off by *R. solani*. However, at higher
concentrations, biochar was ineffective or even increased the disease incidence and severity as compared with the control, forming a U-shape response curve versus biochar concentration, known as hormesis effect. We also observed this effect in experiment 1. In spite of this, greenhouse waste biochar at 3% has also proven to enhance tomato growth and suppression of wilt-inducing chlamydospores (Akhter et al. 2016). This indicates that disease suppression by biochar may vary greatly depending on the studied pathosystem. De Araujo et al. (2021) found that sewage sludge biochar at concentrations below 1% suppressed phytopathogenic fungi while not showing effects on beneficial microorganisms.

In the present study, a thinning in *R. necatrix* hyphae was also observed in the previously cited *in vitro* treatments. Based on this, we hypothesize the following mechanisms to explain this phenomenon: biochar could be inducing metabolic function changes in both fungi, and it could be adsorbing the pathogen’s toxins, thus, making it’s attack less efficient, as studied by Elmer and Pignatello (2011).

Mycorrhiza is known for their ability to aid soil-borne disease suppression, including white root rot (Freire Cruz et al. 2014). most likely via induced plant resistance (Cruz-Paredes et al. 2019; Holland et al. 2019). The present results suggest that the combination of biochar and mycorrhiza has a positive impact on plant growth (Ishii and Kadoya 1994). The combination of rice husk biochar with mycorrhiza showed higher percentages of leaf P, which could indicate that, not only the P in this biochar is more available for the plant, but also, mycorrhiza can solubilize it more efficiently. Similar findings confirming the positive effect of the joint application of biochar and mycorrhiza are also reported by Bakry et al. (2014). Jabborova et al. (2021) also found that the combined application of biochar and mycorrhiza can be used as an efficient biofertilizer to promote plant growth and yield of spinach in field conditions. Regarding the root fresh weight results, mycorrhiza associated with biochar showed better results when compared to some treatments. More specifically, rice husk biochar had a more pronounced relationship with the mycorrhiza. This is most likely due to the fact that rice husk biochar’s carbon is more readily acquired by the mycorrhiza than bamboo biochar’s carbon. Because we used sterile substrate in this experiment, it is likely that the driving forces of those phenomena are not the soil microbiome, but a more direct relationship between biochar and mycorrhiza. Studies have related the ability of mycorrhizal fungi to transfer substantial amounts of nitrogen to their host plant (Leigh et al. 2009; Craine et al. 2009), which could explain the high levels of leaf N in the treatment with only mycorrhiza. Biochar treatments showed the lowest N leaf contents. Akhter et al. (2016) also found that biochar amendment decreased tomato leaf N content, probably due to NH$_4^+$ adsorption by the surface of biochar, even though biochar amended plants had higher yield and fruit quality. Rice husk biochar amendment decreased mycorrhizal colonization when compared to the control treatment. This is likely due to this biochar increasing P% in leaves by enhancing P absorption by the plant. Previous studies have related a negative correlation between P concentration and mycorrhizal colonization (Solaiman et al. 2019; Li and Cai 2021; Yan et al. 2021b), which confirms our results.

4 Conclusions
Biochar amendment successfully suppressed white root rot disease in vivo, while AMF did not aid disease control. Our results indicate that there could be benefits to a joint use of biochar and T. atroviridae for soil-borne disease control, which could be better investigated through a greenhouse experiment involving both. However, in our greenhouse experiment, as we used sterile soil, we did not consider the influence of the soil microbiome. Therefore, in order to better understand the effects of biochar on R. necatrix suppression in a realistic situation, we recommend setting up a field experiment with the joint application of rice husk biochar and T. atroviridae on Japanese apricot trees infected with R. necatrix.

Declarations

Author's contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mateus Oliveira Gomes, Tsubasa Izawa, and André Freire Cruz. The first draft of the manuscript was written by Mateus Oliveira Gomes and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

The authors declare no conflict of interests.

Data availability

The current manuscript has no associated data.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

References


Table

Table 1. Shoot fresh weight, root fresh weight and leaf N of greenhouse Japanese apricot plants. BB = bamboo biochar; BRH = rice husk biochar; Gm = mycorrhiza; C = control. Different lowercase letters within a column indicate significant differences at P = 0.05 among the different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot fresh weight (g)</th>
<th>Root fresh weight (g)</th>
<th>Leaf N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB+Gm</td>
<td>23b</td>
<td>22b</td>
<td>1.77c</td>
</tr>
<tr>
<td>BRH+Gm</td>
<td>26.6a</td>
<td>34.2a</td>
<td>2.39bc</td>
</tr>
<tr>
<td>C+Gm</td>
<td>19.1ab</td>
<td>29ab</td>
<td>3.02a</td>
</tr>
<tr>
<td>C</td>
<td>13.6b</td>
<td>14.3b</td>
<td>2.65b</td>
</tr>
</tbody>
</table>

Figures
Figure 1

In vitro hyphal growth over the course of 3 days, starting at 3 days after inoculation of *T. atroviridae* and *R. necatrix* on a dual culture system amended with rice husk biochar at 0.5%. Ta = *T. atroviridae*; Rn = *R. necatrix*; BRH = rice husk biochar
Figure 2

Photo of the dual culture *in vitro* experiment showing the intersection between the hyphae of the two fungi (halo zone). BRH = rice husk biochar.
Figure 3

White root rot disease severity score in Japanese apricot plants at greenhouse conditions. C = control; Rn = R. necatrix; BRH = rice husk biochar; BB = bamboo biochar; Gm = mycorrhiza; dai = days after inoculation. Scores range from 1 to 5, where 1 = no symptoms and 5 = 100% infection (dead plant).
Figure 4

Percentage of mycorrhizal colonization and leaf P content in greenhouse Japanese apricot plants. BB = bamboo biochar; BRH = rice husk biochar; Gm = mycorrhiza; C = control. Different lowercase letters indicate significant differences at P = 0.05 among the different treatments.
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