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Biochar

RAPID REPORT

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Wood-based biochars produced at low pyrolysis temperatures are good carriers for a *Trichoderma*-based biopesticide

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Abstract

The goal was to investigate biochars' potential as carrier for commercial *Trichoderma*-based biopesticides, facilitating their application in soil or growing media. Thirty-five biochars produced from various feedstocks and pyrolysis temperatures were chemically characterized. Incubation and cold storage tests using a commercial *Trichoderma*-based biopesticide were done. Properties leading to good *Trichoderma* carrier capacity (TCC) are wood-based feedstocks and low pyrolysis temperatures ($p < 0.001$). Multivariate linear regression showed that TCC = exp (23.0 (± 2.21)–1.03 (± 0.25) *pH-H_2O–0.94 (± 0.32) *inorganic carbon–0.10 (± 0.02) *total phosphorus + 0.0005 (± 0.0002) *water-soluble carbon).

Keywords Biocontrol formulations, Carbon, Feedstock, *Trichoderma asperellum* T34

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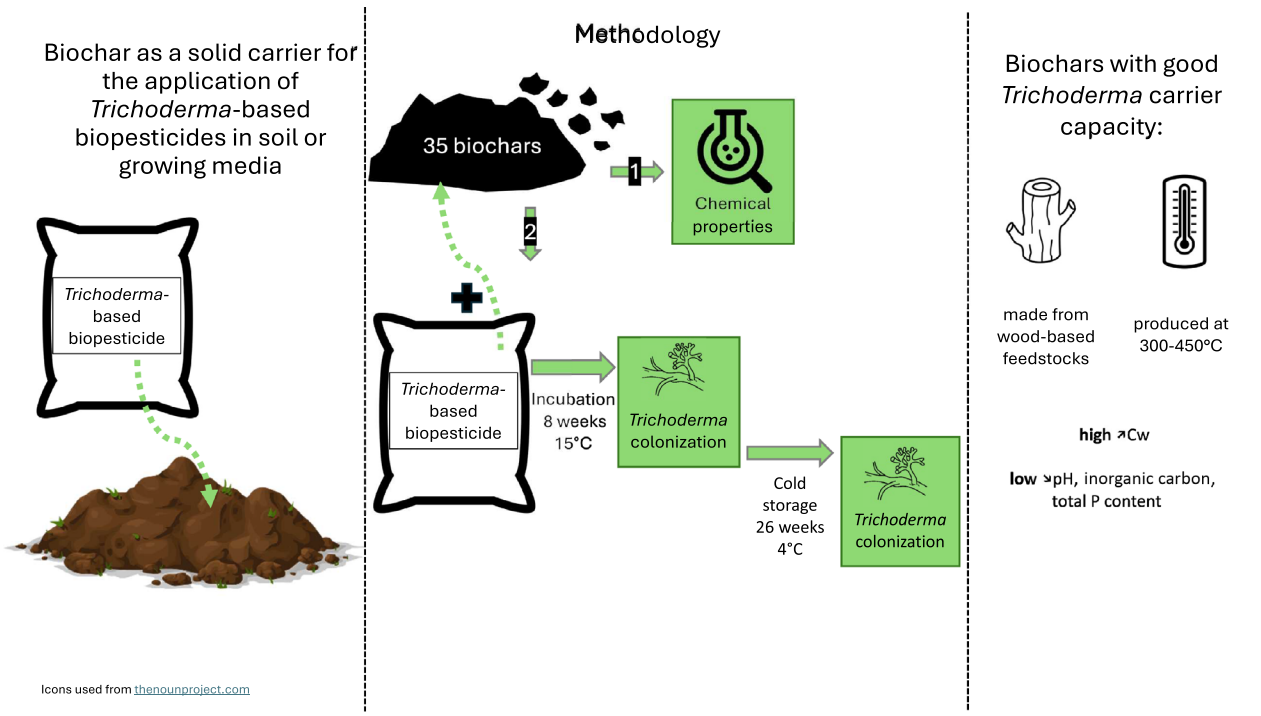
Graphical Abstract



1 Introduction

Biochar is a promising candidate as an inoculant carriers for beneficial micro-organisms such as biocontrol agents (BCA), given its highly porous structure, nutrients naturally derived from the feedstock, and high water and nutrient retention properties (Ajeng et al. 2020; Bamdad et al. 2022). However, biochars are highly variable due to different feedstocks and pyrolysis conditions (e.g. pyrolysis temperature, reactor type and settings) used and there is few knowledge about which biochar characteristics predict their BCA carrying capacity (Bolan et al. 2023).

Trichoderma spp. are widely used as a BCA as they target multiple plant pathogens both directly, through their antagonistic and mycoparasitic activity, and indirectly, by triggering plant defense mechanisms (Benítez et al. 2004; Verma et al. 2007). Two important issues for the biocontrol capacity of *Trichoderma* are to achieve an easy application method and a stable population concentration over time (Joos et al. 2020). To guarantee this for the application in soil or growing media, *Trichoderma* spp. can be inoculated onto organic carriers such as biochar. Organic carriers may not only provide the food and space required for the *Trichoderma,* but may also improve soil or growing media conditions when applied (Pertot et al. 2015; Martinez et al. 2023).

The goal of this research was to investigate the potential of biochars as a carrier for commercial *Trichoderma*-based biopesticides and to predict their *Trichoderma* carrier capacity based on simple or fast measurable properties such as their feedstock, pyrolysis temperature and chemical characteristics. This rapid screening will allow the selection of the most appropriate biochar for use as a solid carrier, facilitating the application of *Trichoderma*-based biopesticides in growing media or soil before planting. Thirty-five biochars, made from various feedstocks at different pyrolysis temperatures, were tested in an eight-week incubation test using a commercial *Trichoderma*-based biopesticide. Next to feedstock and pyrolysis temperatures, the chemical characteristics of the biochars were also correlated with the results of the incubation test. Finally, four biochars were selected for a cold-storage experiment to verify if the selected biochars were also good *Trichoderma* carriers during long time (26 weeks) cold storage.

2 Materials and methods

2.1 Biochars and their chemical characterization

In total, 35 biochars obtained from pyrolysis facilities at Hasselt University (Belgium), ECN > TNO (The Netherlands) and Proininso (Spain) were categorized into three feedstock types (wood, manure and other) and two pyrolysis temperature classes (low = 300–450 °C and high = 600–750 °C) (Table 1). Reactor and pyrolysis conditions can be found in Table S1. In short, the biochars from Hasselt University were produced using a modified pilot-scale rotary kiln reactor. The biochars produced by ECN > TNO were made by the Pyromaat reactor under

Table 1 Feedstock (type) and pyrolysis temperature (class) of the biochars used in this study. *HTT* Highest treatment temperature

Biochar code	Feedstock	Feedstock type	HTT (°C)	HTT class	Reference	Production facility
B1_650_oak	Oak	Wood	650	High	Viaene et al. (2023)	Proininso
B6_400_wood	Wood residues from forestry management	Wood	400	Low	Viaene et al. (2023)	ECN > TNO
B11_300_presscake	Presscake from digestate of dry anaerobic digestions	Other	300	Low	Viaene et al. (2023)	ECN > TNO
B16_650_peat	Peat-based spent growing medium from strawberry cultivation	Other	650	High	Viaene et al. (2023)	ECN > TNO
B17_650_coir	Coir-based spent growing medium from strawberry cultivation	Other	650	High	Viaene et al. (2023)	ECN > TNO
B18_670_wood	Wood residues	Wood	670	High	Viaene et al. (2023)	ECN > TNO
B27_450_chickenmanure	Pelleted organic fertilizer based on chicken manure	Manure	450	Low	Viaene et al. (2023)	Hasselt University
B28_600_chickenmanure	Pelleted organic fertilizer based on chicken manure	Manure	600	High	Viaene et al. (2023)	Hasselt University
B29_750_chickenmanure	Pelleted organic fertilizer based on chicken manure	Manure	750	High	Viaene et al. (2023)	Hasselt University
B30_450_applewood	Apple wood residues	Wood	450	Low	Viaene et al. (2023)	Hasselt University
B31_600_applewood	Apple wood residues	Wood	600	High	Viaene et al. (2023)	Hasselt University
B32_750_applewood	Apple wood residues	Wood	750	High	Viaene et al. (2023)	Hasselt University
B33_450_spstrawpeat	Spent strawberry peat	Other	450	Low	Viaene et al. (2023)	Hasselt University
B34_600_spstrawpeat	Spent strawberry peat	Other	600	High	Viaene et al. (2023)	Hasselt University
B35_750_spstrawpeat	Spent strawberry peat	Other	750	High	Viaene et al. (2023)	Hasselt University
B36_450_coffee	Dried coffee grounds	Other	450	Low	Viaene et al. (2023)	Hasselt University
B37_600_coffee	Dried coffee grounds	Other	600	High	Viaene et al. (2023)	Hasselt University
B38_750_coffee	Dried coffee grounds	Other	750	High	Viaene et al. (2023)	Hasselt University
B39_450_frass	Pasteurized insect frass	Manure	450	Low	Viaene et al. (2023)	Hasselt University
B41_600_frass	Pasteurized insect frass	Manure	600	High	Viaene et al. (2023)	Hasselt University
B42_750_frass	Pasteurized insect frass	Manure	750	High	Viaene et al. (2023)	Hasselt University
B43_450_gwaste	Woody fraction of greenwaste	Other	450	Low	Viaene et al. (2023)	Hasselt University
B44_600_gwaste	Woody fraction of greenwaste	Other	600	High	Viaene et al. (2023)	Hasselt University
B45_750_gwaste	Woody fraction of greenwaste	Other	750	High	Viaene et al. (2023)	Hasselt University
B47_600_shrbark	Shredded bark	Wood	600	High	Viaene et al. (2023)	Hasselt University
B48_750_shrbark	Shredded bark	Wood	750	High	Viaene et al. (2023)	Hasselt University
B49_450_flax	Flax shives (1 batch)	Other	450	Low	Viaene et al. (2023)	Hasselt University
B50_600_flax	Flax shives (1 batch)	Other	600	Low	Viaene et al. (2023)	Hasselt University
B51_450_flax	Flax shives (mix of 2 batches)	Other	450	Low	This study	Hasselt University
B52_450_wood	Wood	Wood	450	Low	Viaene et al. (2023)	Hasselt University
B83_450_gwaste	Greenhouse waste: pruning of raspberry cultivation	Other	450	Low	This study	Hasselt University
B87_450_gwaste	Greenhouse waste: pruning of blackberries cultivation	Other	450	Low	This study	Hasselt University
B88_600_gwaste	Woody fraction of green waste	Other	600	High	This study	Hasselt University
B89_450_gwaste	Greenhouse waste: blackberry roots	Other	450	Low	This study	Hasselt University
B91_450_gwaste	Greenhouse waste: raspberry roots	Other	450	Low	This study	Hasselt University

controlled conditions. The biochar B1_650_oak from Proininso was made by slow pyrolysis at atmospheric pressure, with a residence time in the kiln of 12–18 h at 0% O_2 content.

Biochars were extensively chemically characterized as described in previous research (Viaene et al. 2023; Lataf et al. 2022). For the purpose of the current research, we selected parameters with low correlation among them, including dry matter (DM), fresh bulk density, organic and inorganic carbon (OC and IC), pH-H_2O, electrical conductivity (EC), water-soluble C and phosphorus (C_w and P_w), total P, nitrogen (N), calcium (Ca) content and C:N ratio (Table S2).

3 *Trichoderma* carrier capacity of biochars

3.1 The incubation experiments

The 35 biochars listed in Table 1 were inoculated with the commercial product ASPERELLO® T34 Biocontrol. Asperello T34 is a wettable powder (WP) formulation containing the fungal *Trichoderma asperellum* T34 strain at 1×10^{12} colony-forming units (CFU) kg^{-1}. *T. asperellum* strain T34 is patented under patent No. US 7553657B2 (Trillas Gay & Vilaplana Cotxarrera 2009). Genetic analysis of this strain was recently done using genotype-by-sequencing by Van Poucke et al. (2024).

The inoculation protocol included mixing the biochar with Asperello T34, followed by gently moistening the biochar-Asperello T34 mixture and incubation. Briefly, 50 mg of Asperello T34 was added to 20 g biochar in a plastic bottle. The theoretical *T. asperellum* T34 starting concentration of the biochars was thus about 2×10^6 CFU g^{-1} biochar. Subsequently, to moisten the biochar, distilled water was added based on the DM content of the biochar (see Table 2) and each plastic bottle was covered with parafilm. For each biochar, a negative control was included receiving the same treatment, but without the addition of Asperello T34. All bottles were incubated for eight weeks at 15 °C. In the first four weeks, the bottles were weighed every week. If the weight was less than the original weight, distilled water was added until the weight returned to the original level.

Table 2 Amount of water added based on the dry matter (DM) content of the biochar

DM (%/fresh biochar)	Added mL water/L biochar
< 50	0
50–60	50
60–70	75
> 70	100

Laboratory compacted bulk density was used to convert from weight biochar (g) to volume biochar (L)

In total, eight experiments were done. To check the reproducibility of each experiment, always one biochar (B11_300_presscake) with a known good BCA carrier potential was included as a positive control. At the start of each experiment, the viability of the pure Asperello T34 product was tested. In addition, in one experiment, the pure Asperello T34 product (without biochar) was incubated to test the viability of the pure product after incubation for eight weeks at 15 °C. The enumeration of the *T. asperellum* CFU in the pure product and on the incubated biochar was done as described below (3.3).

3.2 The cold-storage experiment

The cold-storage survival experiment after incubation was done based on Hardy and Knight (2021). Four inoculated biochars (three with good *Trichoderma* carrier capacity and one with medium *Trichoderma* carrier capacity) were selected from the incubation experiment above and stored in closed plastic bottles at 4 °C for 26 weeks (six months). The enumeration of the *Trichoderma* on the cold-stored biochars was done as described below (3.3).

3.3 Enumeration of *Trichoderma* on the incubated and cold-stored biochars

The population of *Trichoderma* on the incubated and cold-stored biochars was enumerated via plating on Trichoderma Selective medium (TSM) as described by Joos et al. (2020). Briefly, 30 ml biochar was placed in a beaker and water was added until 90 ml volume (water + biochar) and this was stirred to reach a homogenous suspension. Subsequently, a serial dilution (1:10, 1:100, 1:1000) was made in duplicate and 100 µl of each dilution was plated on a TSM plate. The plates were then incubated for 5 days in the dark at room temperature, after which the *Trichoderma* colonies per plate were counted. The mean of these plate counts (CFU per plate) was then converted to CFU ml^{-1}. Finally, based on the fresh bulk density of each biochar, the CFU ml^{-1} was converted to CFU g^{-1} biochar. The viability of the pure Asperello T34 product without biochar (at the start of each experiment and after incubation for one experiment) was checked by plating in duplication a serial dilution (see above) of a suspension of 1 g Asperello T34 product in 10 mL sterile water.

4 Data analysis

All statistical analyses were performed using the open-source software platform R (version 4.3.0; R Core Team 2023). First, an explanatory data analysis was performed by visualizing the data from multiple perspectives using

the R package ggplot2 (Wickham 2016) to find the main patterns in the data and to control for deviant patterns. Generalized linear models (GLMs) were used to measure the effect of feedstock type (wood, manure and other) and pyrolysis temperature class (low and high) on the *Trichoderma* carrier capacity of biochars, expressed as the log transformation of CFU. As CFU is a positive count, we used a GLM with negative binomial family, with and without interaction between feedstock type and pyrolysis temperature. Next, as feedstock type and pyrolysis temperature define the chemical characteristics of the biochar, negative binomial GLMs were also used to identify which chemical characteristics explain the *Trichoderma* carrier capacity. Model selection was based on the AIC criterion, and normality of the residuals was inspected using QQ plots and a Shapiro test.

5 Results

5.1 The incubation experiments

For each experiment, the viability of the Asperello T34 product was similar to the theoretical starting concentration (2.0×10^6 CFU added g^{-1} biochar). The results of the biochar used as a positive control (B11_300_presscake) for each experiment was in the range of 1.0×10^6 CFU g^{-1} biochar. The viability of the pure Asperello T34 product decreased about five times during incubation (from 4.7×10^8 CFU g^{-1} product to 9.7×10^7 CFU g^{-1} product). No un-inoculated biochar showed *Trichoderma* growth on the plates.

The *Trichoderma* carrier potential of the biochars varied between 2.0×10^2 CFU g^{-1} biochar (= detection limit) and 1.1×10^7 CFU g^{-1} biochar. Taking the starting concentration of 2.0×10^6 CFU g^{-1} biochar into account, for only one biochar (from flax shives produced at low temperature: B49_450_flax), the number of viable *Trichoderma* CFU increased during the incubation test. Nine biochars showed similar CFU levels after incubation to the starting concentration. Ten biochars were thus good carriers, as they increased or maintained the initial *Trichoderma* population. Similar as for the pure Asperello T34 product, a decrease in CFU up to 5–10 times was found after incubation for almost half of the biochars (n = 16). Nine biochars had a higher decrease (≥ 100 times) in CFU, with five biochars close or under the detection limit.

Statistical analysis showed no interaction between pyrolysis temperature and feedstock. Feedstock type had a significant relationship with the *Trichoderma* carrier capacity. More specifically, wood-based and other non-manure biochars were better carriers than manure-based biochars ($p < 0.001$; Fig. 1A). Furthermore, biochars produced at low pyrolysis temperatures (300—450 °C) were better carriers than biochars produced at high temperatures (600–750 °C) ($p < 0.001$; Fig. 1B).

In a next step, the *Trichoderma* carrier capacity of the biochars was predicted by the chemical characteristics of the biochars. There was a negative relationship with pH-H_2O, IC and total P and a positive relationship with C_w according to the following formula:

Trichoderma carrier capacity = exp (23.0 (± 2.21)–1.03 (± 0.25) *pH-H_2O–0.94 (± 0.32) *IC–0.10 (± 0.02) *total P + 0.0005 (± 0.0002)* C_w) with IC is expressed as %IC/DM, total P is expressed as g P/kg DM and Cw is expressed as mg C/L biochar.

5.2 The cold storage experiment

Cold storage at 4 °C of four inoculated biochars (selected from the incubation experiment) for 26 weeks revealed a similar range of *Trichoderma* concentrations per gram biochar between eight weeks of incubation and eight weeks of incubation combined with 26 weeks of cold-storage (Table 3). This indicates that the selected biochars were also good *Trichoderma* carriers during cold storage.

6 Discussion

Biochar has recently been proposed as an innovative solid carrier for *Trichoderma*-based formulations (Martinez et al. 2023). However, biochar characteristics that predict the *Trichoderma* carrier capacity have not been determined before. Moreover, previous research on the microbial carrier capacity of biochars was mainly done using wood residues and maize wastes as feedstocks (Bamdad et al. 2022), whereas this is the first time that the microbial carrier capacity of biochars with various feedstocks including consumer waste such as coffee grounds, spent growing media and manure (chicken, insect frass) has been tested.

Selected properties for biochars to show good *T. asperellum* T34 carrier capacity were wood-based feedstocks produced at low pyrolysis temperatures (300–450 °C). Desirable chemical properties were high C_w, low pH, IC and total P content. Higher pyrolysis temperatures result in more recalcitrant biochars (less functional groups) and are thus much harder for microorganisms to metabolize (Bolan et al. 2023; Janu et al. 2021). This is also reflected in the C_w content of the biochars which is linked to the pyrolysis temperature. Generally, C_w tends to decrease with increasing pyrolysis temperature. This is because higher temperatures during pyrolysis result in more extensive carbonization and conversion of organic compounds into stable forms, reducing the solubility of carbon in water (Bolan et al. 2023). The higher the availability of soluble C, the better the growth of *T. asperellum* T34. Total P and IC contents are linked to feedstock type. More specifically, manure-based biochars have

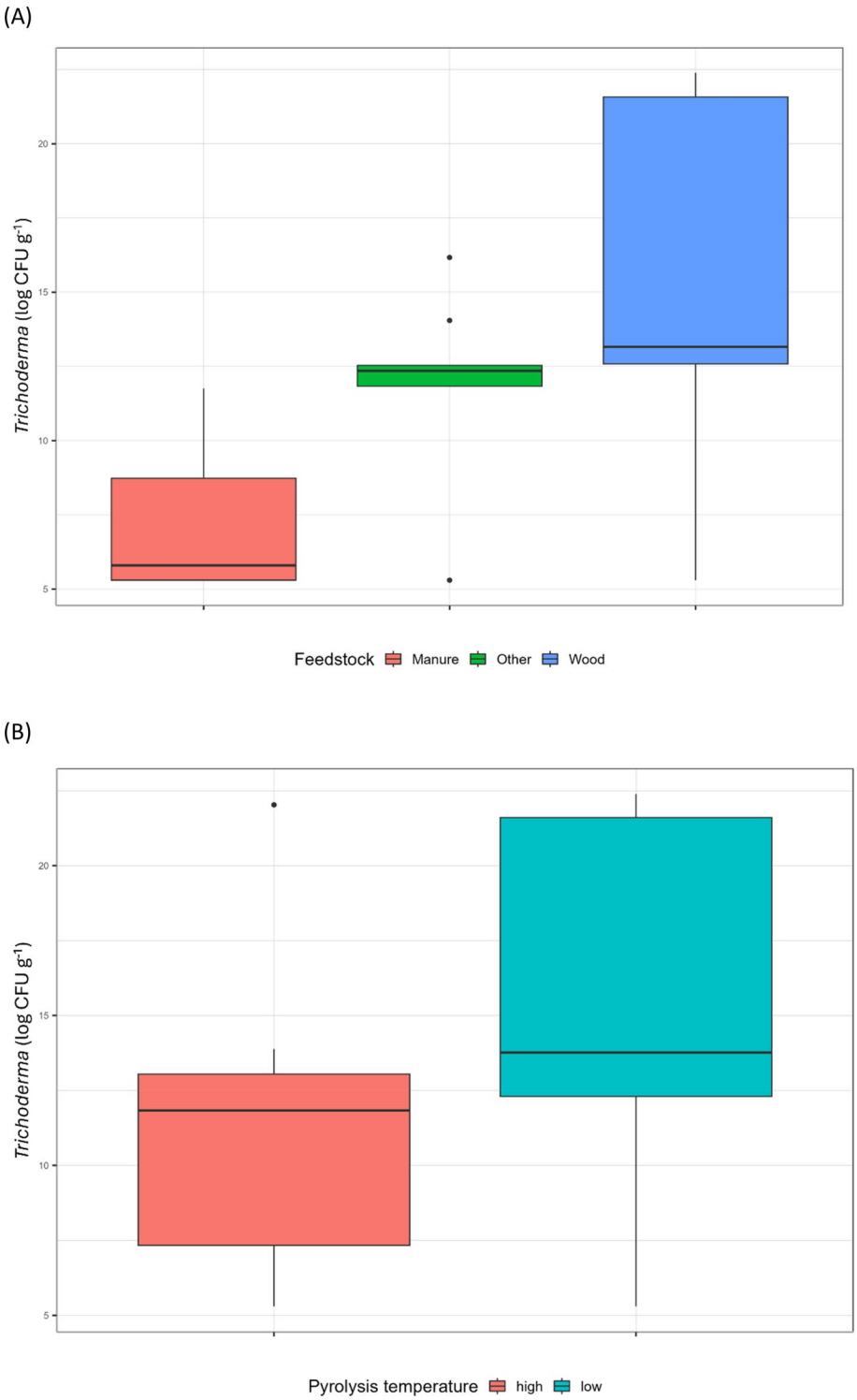


Fig. 1 *Trichoderma* carrier capacity (log CFU g^{-1} biochar) in relation to feedstock type (**A**) and pyrolysis temperature (**B**)

higher total P and IC contents than wood-based (Viaene et al. 2023) and therefore they are not a good carrier for *T. asperellum* T34. The inorganic C content and the acid-buffering capacity of the biochar are important characteristics in relation to the pH-related effect of biochar. The acid-buffering capacity of biochar, i.e., its capacity

Table 3 *Trichoderma* carrier capacity (CFU g^{-1} biochar) of 4 biochars after incubation at 15 °C for 8 weeks and after incubation for 8 weeks at 15 °C and cold storage at 4 °C for 26 weeks

Biochar	Incubation for 8 weeks at 15 °C	Incubation for 8 weeks at 15 °C + storage for 26 weeks at 4 °C
	CFU g^{-1}	
B51_450_flax	1.1×10^6	2.1×10^6
B52_450_wood	1.3×10^6	2.3×10^6
B88_600_gwaste	5.7×10^3	2.5×10^4
B89_450_gwaste	2.4×10^6	1.6×10^6

to maintain a pH in the alkaline range and act as a liming agent, is strongly positively related to the inorganic C of the biochar (Lataf et al. 2022). Higher IC contents and the related high pH values may result in less optimal conditions for biocontrol fungi. pH is linked to both pyrolysis temperature and feedstock as the pH increases with increasing pyrolysis temperature and wood-based biochars have generally a lower pH (< 8.5) than manure-based (> 9.5) biochars (Bolan et al. 2023; Viaene et al. 2023).

Selected biochars were able to keep their *Trichoderma* carrier capacity during 6 months of cold storage at 4 °C. It is very common for microbial products, including for Asperello T34, to be stored at 4 °C (see technical sheet ASPERELLO® T34 Biocontrol®). This may indicate that well-selected biochars may also preserve the shelf-life of BCA products. The potential of biochar to impact rhizobial shelf life and survival has been reported before (Shabir et al. 2023), but to our knowledge this is the first report about shelf-life and longtime survival of fungal inoculants.

We selected Asperello T34, a formulated product containing the strain *T. asperellum* T34, as a model for *Trichoderma*-based biopesticides. Under its current use, Asperello T34 is suspended in water and applied as a suspension to the growing medium or soil. As the recommended dose is very low (10 g m^{-3}, see technical sheet ASPERELLO® T34 Biocontrol®), adding and mixing low amounts of Asperello T34 to soil or growing media is a challenge. Therefore, a solid premix of Asperello T34 and biochar is useful to facilitate the application in growing media or soil before planting.

Further research is necessary to test whether the results with this formulated strain can be extrapolated to other *Trichoderma* strains and other formulations. Further research should also focus on relating our simple and fast measurable chemical properties with physical properties such as surface area, volume and pore diameter (measured by Brunauer–Emmett–Teller and Barrett-Joyner-Halenda analysis), as these physical properties are reported to be important for microbial inoculation of biochars (Jaafar et al. 2015). Moreover, next to ensuring and predicting the survival of *Trichoderma*-based biopesticides on biochars, further research should test the biocontrol efficacy of the inoculated biochars in greenhouse tests or field trials.

In conclusion, for use as a solid carrier for *Trichoderma*-based biopesticides, we suggest to use biochars made from wood-based materials produced at low pyrolysis temperatures (300–450 °C) and/or biochars with high C_w and low pH, IC and total P content.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-024-00368-5.

Additional file 1.

Additional file 2.

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Author contributions
BV, AC & JD: funding acquisition and supervision of the project. BV, JD, LJ & SF: design and supervision of the experiments. KM: conduction of the inoculation experiments and calculations. JV: statistical analysis. JD: wrote the first draft and finalized the manuscript. All authors contributed to the writing of the manuscript and approved submission.

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Data availability
The data that support the findings of this study are available upon request.

Declarations

Competing interests
All authors declare they have no financial interests.

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