Midas

MARGINAL LANDS, INDUSTRIAL CROPS AND INNOVATIVE BIO-BASED VALUE CHAINS

Deliverable D4.11

Biochar and wood vinegar production and characterization for agriculture

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Executive summary

This deliverable reports on the activities carried out under Task 4.2.4 "Biochar," led by RE-CORD, covering the period from Month 1 to Month 36. The primary objective of this task is the production and characterization of biochar and wood vinegar (pyrolysis condensate), assessing their feasibility for agronomic and industrial applications within the MIDAS value chains.

Chapter 1 provides a comprehensive definition of biochar, distinguishing it from charcoal and detailing production technologies (pyrolysis vs. gasification) and plant scales. The section analyses biochar's fundamental properties—such as stable carbon content (H:C ratio), porosity, and potential contaminants—and outlines the current regulatory framework. Particular attention is given to the EU Fertilising Products Regulation (FPR 2019/1009), the European Biochar Certificate (EBC) standards, and the emerging Carbon Removal and Carbon Farming (CRCF), positioning biochar as a strategic tool for Carbon Dioxide Removal (CDR). Then, the document reports on RE-CORD's activities in Task 4.2.4: the production of biochar and wood vinegar, i.e. the aqueous fraction of condensates. The activities are presented in dedicated chapters: lab scale carbonization of lignocellulosic and bagasse materials; demo scale carbonization of woody biomass for agronomic field trials; a preliminary investigation of other potential biochar agronomic applications (e.g. mulch films, seed coating); wood vinegar pilot-scale production and related tests as a pre-sowing seed primer, after dilution.

- **Chapter 2: Lab scale carbonization of lignocellulosic and bagasse material**

Chapter 2 presents the results of the laboratory-scale carbonization campaign at 550°C on lignocellulosic residues and bagasse feedstock. The characterization results confirmed that Guayule bagasse, Crambe (whole plant and straw), Miscanthus, and Yellow Melilot are excellent feedstocks, producing biochar potentially fully compliant with EU FPR and EBC quality standards. Safflower hulls presented a Zinc content slightly above the EBC limit for organic agriculture, though potentially suitable for conventional use.

Lab scale carbonization of lignocellulosic residues	
Target	To produce and characterize 8 biochar from different feedstocks, for agronomic purpose.
Method	Samples were produced at lab scale, then characterized following EU FPR 1009/2019 and EBC quality standard.
Result	High quality char was obtained both from woody and herbaceous residues and bagasse, in particular: guayule bagasse, crambe (two different sources), miscanthus, yellow melilot, hemp and safflower straw. They all demonstrated excellent biochar characteristics based on the analyses. The Zinc (Zn) content in the Safflower hulls would exclude the specific product from organic agriculture, according to the limit imposed by the EBC (European Biochar Certificate), although the exceedance was minimal. Biochars with a higher specific surface area and a higher potential water and nutrient retention are the ones produced from *Miscanthus (MxG)* and Yellow Melilot.

- **Chapter 3: Demo scale carbonization of woody biomass for agronomic field trials**

Chapter 3 details the demo-scale production carried out using the continuous "PYROK" rotary kiln. The activity successfully achieved the target production of 120 kg of woody biochar, which was characterized, confirmed as compliant for use as a soil improver, and shipped to project partners (Novamont, UNICT, CIEMAT, CRES) for the agronomic field trials scheduled in Task 2.2.3.

Demo scale carbonization of woody biomass for agronomic field trials	
Target	The targeted production is 120 kg of biochar on a dry basis. 30 kg/partner, 4 partners.
Method	Samples were produced at demo scale, through slow pyrolysis, in PYROK plant. The biochar produced was fully characterized, following EU FPR 1009/2019, to check its compliance as soil improver.
Result	All the parameters confirm the compliance of the material as an organic soil improver for agronomic applications.

- **Chapter 4: Other potential biochar applications, preliminary investigation**

Chapter 4 explores alternative high-value applications. Biochar was micronized <40 µm and evaluated as a bio-based substitute for carbon black in mulch films (in collaboration with Novamont). Additionally, biochar-based seed coating was tested on maize, sorghum, and beans. Preliminary germination tests yielded mixed results: while neutral or positive effects were observed for maize and sorghum, the negative impact on beans suggests a sensitivity to the binder (Polyethylene glycol, PEG) or the coating process, indicating the need for further optimization using biological binders like starch.

Other potential biochar applications, preliminary investigation	
Target	The target was the investigation of innovative potential uses for biochar agronomic applications.
Method	• 2 kg of biochar was produced (PYROK demo plant), milled and sieved at 40 µm; its characterization followed EU FPR 1009/2019. • Biochar based seed coating was explored coating 3 different seed species (maize, sorghum and bean), with a binder (Polyethylene glycol, PEG). A preliminary germination test in trays was conducted.
Results	• The material resulted compliant with EU FPR 1009/2019 for agronomic use. The material was sent to NOVAMONT for its evaluation as a bio-based substitute for Carbon Black masterbatch. The particle size distribution was excessively large for the material to be used, as shipped. This could necessitate a further reduction step, which could present a challenge regarding energy demand. • Seeds were successfully coated with and without a binder. The germination preliminary test suggests a positive baseline effect for sorghum and maize under non-stress conditions, contrasting with

	the negative response of the bean. The detrimental effect observed on the bean may be related to the specific biochar or the binder (PEG) used, reinforcing the need to use alternative products, possibly biological such as starch, which are less likely to negatively impact germination.

- **Chapter 5: Wood vinegar production**

The aqueous fraction resulting from slow pyrolysis, commonly referred to as wood vinegar, was produced at pilot scale using the SPYRO facility. Following collection and separation from the oil phase, the wood vinegar was thoroughly characterised before being dispatched to project partners (Task 2.2.3) for evaluation of its suitability as a bio-stimulant, with a particular focus on its application as a seed primer prior to sowing.

Wood vinegar production	
Target	Production and characterization of slow pyrolysis condensate from woody biomass. In particular, the aqueous phase separation, to be characterized as a potential biostimulant when diluted.
Method	Sample were produced at pilot scale, through slow pyrolysis, in SPYRO pilot plant. Condensate and the other co-products were characterized.
Result	The condensate aqueous phase sample was highly acidic, with a low pH of 2.77, and contained significant levels of TOC (65719 mg/L). Elemental analysis confirmed its high-water content (~75%wt wb) and high overall C and O content on a dry basis. Key compounds identified included a high concentration of Acetic Acid (95.99 mg/L), confirming its potential as a biostimulant source, alongside other measurable organic compounds like Methanol, Glycerol, Furfural, and various phenolics. The measured HHV confirmed a substantial residual energy content. When diluted, potential inhibitory effects can be avoided while maintaining an interesting content of acetic acid (e.g. at 1 mM).

- **Chapter 6: Wood vinegar as a seed primer**

Chapter 6 presents germination tests, conducted in climatic chamber with different seeds typologies to evaluate the wood vinegar performance. A specific dilution protocol (targeting 1 mM acetic acid) was developed to mitigate phytotoxicity. Germination trials under water stress revealed species-specific responses: *Castor Bean* benefited significantly from the treatment, showing increased germination and reduced variability; *Sorghum* showed a neutral response; whereas *Safflower* proved sensitive, with the treatment negatively affecting germination rates.

Wood vinegar production	
Target	The targeted production of the diluted wood vinegar was 5 litres, to be shipped to partners (Task 2.2.3) as a bio-stimulant product to be tested. Another target was the protocol definition to dilute the wood vinegar as biostimulant in presowing seed imbibition, to promote germination

	under stress conditions. Germination tests were conducted in climatic chamber with different seeds typologies to evaluate its performance.
Method	The wood vinegar was diluted to reduce potential inhibitory effects, maintain a target of acetic acid of 1 mM concentration. Presowing seed imbibition tests were performed with 3 different seed typologies. Germination tests were conducted in controlled environment with and without water stress.
Result	• A protocol was sent to partners to prepare the diluted wood vinegar at 1 mM concentration of acetic acid. Based on the germination trials, the three tested species demonstrated highly variable responses to the imbibition treatments and water regimes, underscoring the necessity of a species-specific protocol optimization. • Castor Bean, despite its low overall germination rate (approx. 20%), was the only species where wood vinegar imbibition produced a clear benefit, yielding the best performance (and lowest variability) under water stress, suggesting enhanced seed vigor and initial stress tolerance. In summary, the efficacy of the pyrolysis-derived solution ranges from beneficial (Castor Bean) to neutral (Sorghum) and detrimental (Safflower).

- **Conclusions**

The activities conducted in Task 4.2.4 have successfully identified suitable feedstocks, optimized production protocols, and delivered the materials necessary for the project's agronomic validation phase. The findings highlight the importance of a "tailor-made" approach, where biochar and wood vinegar applications are specifically matched to crop tolerance and soil needs to maximize benefits and ensure safety.

In *Table 1* the scheduled production of materials, the delivery dates, the partners involved and related linked activity are reported.

Table 1: Expected production of biochar and wood vinegar.

Product	Amount expected	Delivery date	End user	Linked to activity
Biochar for field trials	120 kg	delivered in November 2023	NOVAMONT, UNICT, CIEMAT and CRES	2.2.3
Biochar for mulch films	2 kg	delivered in February 2024	NOVAMONT	4.2.2
Diluted wood vinegar	5 L	delivered in December 2024	NOVAMONT, UNICT, CIEMAT and CRES	2.2.3

Summary

1. Introduction: biochar

Terms like biochar, pyrochar, and charcoal are often used interchangeably, which may cause confusion. In general, biochar is defined as a vegetable carbon produced by the carbonization process, but intended for specific end-uses. Hagemann and Schmidt distinguished biochar from charcoal: "Biochar is a porous, carbonaceous material that is produced by pyrolysis of plant biomasses and is applied in such a way that the contained carbon remains stored as a long-term C sink or replaces fossil carbon in industrial manufacturing. It is not made to be burnt for energy generation"[1]. According to Lehmann and Joseph biochar use is focused on environmental management and carbon sequestration: "[…] it (i.e., biochar) distinguishes itself from charcoal and similar materials […] by the fact that biochar is produced with the intent to be applied to soil as a means of improving soil productivity, carbon (C) storage or filtration of percolating soil water"[2]. Of course, what is called biochar has also a wide range of different product characteristics (e.g., ash content, porosity structure, stability grade, functional groups in its surface, ion exchange capacity, sorption capacity, etc.) interesting for a lot of potential end uses, different from the classic ones above mentioned.

1.1 Production and technology status

Biochar is the solid carbonaceous material originated from the thermochemical conversion of organic matter via pyrolysis or gasification, the other products being permanent gases and condensable vapors. While there are some similarities in the properties of biochar from slow pyrolysis and gasification process, these do not extend to the viability of the processes for their integration in industrial settings where biochar use is pivotal to the large-scale introduction of biogenic and recovered carbon in energy intensive, hard-to-abate industries, because slow pyrolysis targets biochar as the main product, whereas gasification produces char incidentally.
Globally, the biochar production encompasses different scales and complexities, from rudimentary batch earth mounds for household needs, to large continuous industrial systems that can match the demand of steel making plants, for example in Brazil. Mode of operation, construction material, feedstock size, portability, biomass loading mode and heating method are amongst the criteria used to classify biochar production technologies and reactors. Depending on how energy is supplied to the feedstock, the process can be either allothermal or autothermal. In the allothermal process, an external energy source, usually the combustion of pyrogas (gases and vapors), provides heat to the reactor. In the autothermal process, the energy need is supplied by partially oxidizing the feedstock, typically via air injection. In general, all commercial processes are operated at ambient pressure to contain costs and limit plant complexity, however the benefit of operating at elevated pressure to move closer to the maximum theoretical yield have been validated in the past up to the pilot scale. Drawing on reactor technology, one might find the following types of reactors[3,4]:

- Kilns, traditional technology comprising batch autothermal reactors adopted, generally without recovery of pyrolysis gas;

[1] EBC, "Guidelines for biochar production for a sustainable production of biochar," Frick, Dec. 2022.
[2] "Biochar for Environmental Management," Biochar for Environmental Management, Feb. 2015, doi: 10.4324/9780203762264/BIOCHAR-ENVIRONMENTAL-MANAGEMENT-JOHANNES-LEHMANN-STEPHEN-JOSEPH.
[3] M. Garcia-Perez, T. Lewis, and C. Kruger, "Methods for Producing Biochar and Advanced Biofuels in Washington State Part 1: Literature Review of Pyrolysis Reactors," Pullman, WA, 2011.
[4] J. A. Garcia-Nunez et al., "Historical Developments of Pyrolysis Reactors: A Review,". Energy and Fuels, vol. 31, no. 6, pp. 5751–5775

- Retorts, mostly allothermal reactors able to convert pile-wood or wood logs over 18 cm in diameter and longer than 30 cm;
- Converters, reactors able to convert small-sized feedstock, like wood chips.

1.2 Biochar main properties

A wide variety of factors contribute on the determination of the characteristics of biochar, first and foremost the process adopted for its production and the type of biomass used. These parameters greatly affect the functionality of the obtained biochar, mainly defining its physico-chemical composition and morphological structure.
Biochar mass yield (the solid fraction resulting from the thermochemical conversion of biomass through the pyrolysis process) depends strictly from the feedstock characteristics, the plant used and the process parameters chosen (heating rates, maximum temperature and residence times)[1,2]. The mass yield of high-quality char is commonly about 30 % by dry weight, using these process parameters and lignocellulosic feedstock[3], while minimising the production of liquid and gaseous products.

1.2.1 Highly stable organic carbon content

When compared with the starting biomass, biochar is characterized by a higher carbon content and a lower presence of oxygen and hydrogen. These characteristics outline the high levels of both chemical and biological stability of the biochar. Through carbonization reactions at pyrolysis temperatures, typically between 550°C and 600°C, a **durable form of carbon** is created. This process is key to preventing a significant portion of the biomass's carbon from being rapidly returned to the atmosphere by microbial activity in soil[5,6].
As reported by Leng et al., biochar **Fixed Carbon** is closely related to stable C content, it represents the solid residue that remains after heating the material (at 900°C) in inert atmosphere[7]. Thus, fixed carbon is a parameter that can be preliminary used to evaluate the quality of biochar obtained and to evaluate the potential recalcitrant carbon content of the product; indeed, considering only the Total Carbon content could lead to misinterpretation, because this parameter includes some labile organic carbon. Calvelo Pereira et al. showed how the thermogravimetric analysis (TGA) can be a suitable and practical mean to evaluate both the stable and the labile carbon fractions (respectively fixed carbon and volatile matter content of biochar)[8,9].
The **H:C molar ratio** is a parameter commonly used for gauging product stability. Materials with low H:C molar ratio values, like anthracite and graphite, are more stable and resistant to degradation

[5] Chiaramonti D, Lehmann J, Berruti F, et al. Biochar is a long-lived form of carbon removal, making evidence-based CDR projects possible. *Biochar*. 2024;6:81. doi:10.1007/s42773-024-00366-7.
[6] Chiaramonti D, Berruti F, Lehmann J, et al. Biochar and its impact on the carbon cycle. *Biomass Bioenergy*. 2025;203:108365.
[7] Leng, L., Huang, H., Li, H., Li, J. & Zhou, W. Biochar stability assessment methods: A review. *Science of the Total Environment* vol. 647 210–222 Preprint at https://doi.org/10.1016/j.scitotenv.2018.07.402 (2019).
[8] Calvelo Pereira, R. *et al.* Contribution to characterisation of biochar to estimate the labile fraction of carbon. *Org Geochem* **42**, 1331–1342 (2011).
[9] Brassard, P., Godbout, S. & Raghavan, V. Soil biochar amendment as a climate change mitigation tool: Key parameters and mechanisms involved. *Journal of Environmental Management* vol. 181 484– 497 Preprint at https://doi.org/10.1016/j.jenvman.2016.06.063 (2016).

than materials with high H:C values (such as uncharred biomass)[10]. According to the European Biochar Certificate (EBC) and the International Biochar Institute (IBI), values over 0.7 suggest non-pyrolytic chars or sub-optimal pyrolysis[11,12].

Random Reflectance (Ro) is a petrographic method that has been used for years to measure the percentage of light reflected from a polished surface of a carbonaceous material. It is a fundamental indicator of the material's thermal maturity or degree of carbonization. The methodology[13] to assess the fraction of inertinite, the most stable form of carbon present in the Earth's crust, within biochar was recently developed by Prof. Hamed Sanei of Aarhus University (DK). In the context of biochar, inertinite identifies the most stable and non-reactive component of the material.

1.2.2 Porous structure

Porosity is one of the most critical characteristics of biochar, largely determining its potential applications. The internal structure of the feedstock, under specific production process condition, determines the porous structure of the biochar after the carbonization, in terms of dimension and distribution of the solid particles size and, hence, in terms of porous network and surface area. The porosity structure of biochar is a crucial factor that influences the water and nutrient retention and their plant availability. In general, biochar can possess a specific surface area on the order of hundreds of m^2/g, and a developed porous structure, ranging from **micropores** (about 1-2 nm) to **macropores**, in the order of microns. A thorough analysis of porosity, which includes measuring the volume, distribution, and shape of the pores, is essential for understanding how the material will interact with its environment. Biochar highly macroporous performs excellently as a "carrier," serving as a support for beneficial microorganisms or nutrients, thereby improving soil fertility and health. Conversely, if its porous structure is mainly microporous, biochar can act as an effective "filter" for water or air purification, adsorbing pollutants and unwanted substances. It is precisely in the study of porosity that the fundamental difference between biochar and activated carbon emerges. Although both are produced from the pyrolysis of organic material, the production process for activated carbon includes an additional step, activation, which creates an extremely developed network of micropores. The specific surface area value (obtained through **BET analysis**) is a parameter to be investigated because it can help to preliminary investigate the potential adsorption capacity of the biochar produced.

1.2.3 Contaminants

In general, thermal processing of biomass concentrates most of the inorganics and metals, including heavy metals, in the solid product. These substances are generally inhibitory or toxic toward microorganisms or living organisms in general; besides, also industrial applications as fossil coal substitute might require meeting specific thresholds on the content of inorganics and metals, thus further processing of char e.g. by leaching might be needed to accommodate the end-user requirements. Other contaminants that might be present in the char include organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), dioxins (PCDD/Fs), volatile organic compounds (VOCs), persistent free radicals (PFRs) and other.

[10] Budai et al. (2013). Biochar Carbon Stability Test Method: An assessment of methods to determine biochar carbon stability. 10.13140/RG.2.2.16359.42402.

[11] "EBC guidelines & documents." https://www.european-biochar.org/en/ct/2-EBC-guidelines-documents

[12] "BIOCHAR STANDARDS - International Biochar Initiative." https://biochar-international.org/standard-certification-training/biochar-standards/

[13] Assessing biochar's permanence: An inertinite benchmark. H Sanei, A Rudra, ZMM Przyswitt, S Kousted, MB Sindlev, X Zheng, ... International Journal of Coal Geology 281, 104409

1.3 Potential end-uses

The porous structure of the biochar makes it potentially suitable for a range of uses, including acting as an adsorbent against a wide range of compounds, in addition to water.
Biochar is capable of retaining a variety of substances, including gases, which makes it valuable in odour filtration, as well as both polar and non-polar liquids. This versatility significantly expands its range of industrial applications, with growing interest particularly in the pharmaceutical sector.
The combination of chemical stability and developed porosity may lead to many other potential uses, especially in contexts where its beneficial impact to the microbiological dynamics could be exploited. In this field, recent research focused on evaluating the effect of using biochar in anaerobic digestion processes for organic waste recover, that aimed to produce compost or digestate as by-product of the biogas production.
It has been demonstrated that the biochar provides beneficial effects on both processes, from different points of view (e.g., reducing composting period, accelerating microbiological stabilization, improving biogas yield, reducing pollutants emissions, adsorbing plant-nutrient, and others); such beneficial activities could be mainly related to the adsorbent effect of the biochar and to the improvement of the microbial population involved in relative bioprocesses.
Moreover, due to its high surface area, porosity and mineral content, biochar plays an important role as an adsorbent, to purify air and water from contaminants, or as a catalyst for both tar removal from syngas and for biofuel production. Biochar, indeed, is recognized to be able to adsorb heavy metals, organic contaminants, nitrogen and phosphorus contaminants, and other pollutants from aqueous solutions, with performance often comparable to those of the activated carbons[14]. Lastly, biochar has been used as catalyst in the transesterification and esterification processes for biodiesel production[15,16].

1.3.1 Agronomic use of biochar

The agronomic use of biochar has recently attracted significant interest. However, the concept of applying stable carbonized material to the soil to enhance fertility is ancient; the most representative example of this technique is the so-called "Terra Preta do Índio." The principal agronomic benefits of applying biochar to agricultural land are related to the improvement of soil fertility through enhanced physical, chemical, and biological properties, including: improved mechanical structure, density, and texture; increased porosity and aeration; enhanced water retention capacity; increased pH in acidic soils; elevated cation and anion exchange capacity (CEC and AEC); supply of nutrients and reduced nutrient leaching; greater nitrogen cycling efficiency; provision of recalcitrant organic carbon matrix; and provision of an ideal habitat for microbial development.
Regarding field application rates, available data are numerous but highly heterogeneous. The optimal quantity depends on the specific type of biochar used, the pedological and climatic characteristics of the intervention site, and the intended crop. Consequently, while regulatory standards exist concerning the characteristics biochar must possess for agronomic application, there is currently a perceived lack of comprehensive guidelines for its correct application to the soil.

[14] A. K. Sakhiya, A. Anand, and P. Kaushal, “Production, activation, and applications of biochar in recent times,” Biochar 2020 2:3, vol. 2, no. 3, pp. 253–285, May 2020, doi: 10.1007/S42773-020-00047-1.
[15] Z. Fu, H. Wan, X. Hu, Q. Cui, and G. Guan, “Preparation and catalytic performance of a carbon-based solid acid catalyst with high specific surface area,” Reaction Kinetics, Mechanisms and Catalysis, vol. 107, no. 1, pp. 203–213, Oct. 2012, doi: 10.1007/S11144-012-0466-9/FIGURES/5.
[16] M. Li, Y. Zheng, Y. Chen, and X. Zhu, “Biodiesel production from waste cooking oil using a heterogeneous catalyst from pyrolyzed rice husk,” *Bioresour Technol*, vol. 154, pp. 345–348, Feb. 2014, doi: 10.1016/J.BIORTECH.2013.12.070.

The most significant effects of biochar use are observed in arid and/or marginal soils, such as those that are prevalent in the Mediterranean area. Biochar application enhances the resistance and resilience of these soils, improving their ability to recover integrity following an external disturbance. The use of biochar in agriculture also offers another compelling potential advantage: if managed correctly, it can lead to an economy that is not only net zero, but actually carbon negative. During its life cycle, plant biomass captures CO_2 from the atmosphere, storing it as carbon. The process of carbonization converts a significant portion of this carbon into a stable form. Long-term carbon storage in soils thus prevents its mineralization back into CO_2, thereby limiting and counteracting the greenhouse effect and subsequent climate change progression. Nevertheless, another role biochar plays in reducing the climate carbon footprint is its use as a viable substitute for peat, which is a fossil-derived material widely used in the nursery industry.

1.4 Biochar regulatory framework

1.4.1 Biochar characterization following EU FPR 2019/1009

The **EU Regulation 2019/1009** of 5th July 2019 sets the rules for the suitability of the biochar toward the market of fertilising products. The modifications (applied from 16 July 2022) to Annexes II, III and IV of the EU Regulation included the possibility to classify pyrolysis and gasification materials (biochar) as Component Material Category n.14 (CMC 14). Indeed, the regulation includes provisions for EU fertilising products that contain requirements at two levels in accordance with their intended function ('Product Function Category', PFC), and for the component materials contained in the EU fertilising product ('Component Material Categories', CMC). Specific requirements for CMC 14 (biochar) refer to organic pollutants content, such as PAH-16 (polycyclic aromatic hydrocarbons), PCDD/F (dioxins and furans) and PCB (polychlorinated biphenyls), including also Cl^- (chloride) and Tl (thallium), as shown in the following table.

Table 2: Biochar compliance with EU regulation n.2019/1009 for fertilising products, as CMC 14.

Parameter	PFC 3(A) threshold
PAH16 (mg kg^{-1} db)	< 6.0
PCDD/F (ng $_{WHO\ toxicity\ equivalents}$ kg^{-1} db)	< 20.0
PCB non-dioxin like (mg kg^{-1} db)	< 0.8
Cl^- (g kg^{-1} db)	< 30.0
Tl (mg kg^{-1} db)*	< 2.0
H/C_{ORG} molar ratio**	< 0.7

*Reg. (EU) 2019/1009: with pyrolysis additives >5% of loaded fresh mass
** Reg. (EU) 2019/1009: on dry and ash-free fraction for material with a C_{ORG} content <50%

CMC 14 can be used alone or combined with other CMCs to constitute a PFC. The Product Function Categories n.3A and n.4, for example, permit to characterise a product as soil improver (PFC 3A) or growing medium (PFC 4). These classifications included thresholds for heavy metals content, such as Cd (Cadmium), Cr VI (Hexavalent Chromium), Hg (Mercury), Ni (Nickel), Pb (Lead), As (Inorganic Arsenic), Cu (Copper) and Zn (Zinc), as shown in the following table.

Table 3: Biochar compliance with EU regulation n.2019/1009 for fertilizing products, as PFC 3(A).

Parameter	PFC 3(A) threshold
Organic Carbon (%wt db)	> 7.5
Dry matter (%wt db)	> 20.0
Cadmium (mg kg^{-1} db)	< 2.0
Hexavalent Chromium (mg kg^{-1} db)	< 2.0
Mercury (mg kg^{-1} db)	< 1.0
Nickel (mg kg^{-1} db)	< 50.0
Lead (mg kg^{-1} db)	< 120.0
Inorganic Arsenic (mg kg^{-1} db)	< 40.0
Copper (mg kg^{-1} db)	< 300.0
Zinc (mg kg^{-1} db)	< 800.0
Salmonella spp. (CFU/25g)	absent
Escherichia coli or *Enterococcaceae* (CFU/g)	< 1000

In conclusion, biochar that complies with the EU FPR requirements, is a very clean and safe vegetable carbon to be used for agronomic purposes. The high carbon content, resistant to thermochemical and biological degradation and only marginally subject to mineralisation by microorganisms, coupled with the water holding capacity and the nutrient retention ability, provides an interesting solution for the application of the product as an effective soil improver and, at the same time, a potential innovative carbon capture and storage strategic solution (as indicated in the IPCC report released in 2019[17]) able to persist in soil for decades to millennia.

1.4.2 Product quality standards

In the emerging biochar industry, the importance of international quality standards has grown exponentially to ensure product safety, application reliability, and traceability. These standards, such as the International Biochar Initiative (IBI) Standard, the European Biochar Certificate (EBC), and more recently the World Biochar Certificate (WBC), serve to protect consumers and farmers by providing a clear, science-based regulatory framework. Their primary function is to certify that biochar is free from contaminants, produced sustainably, and possesses the stated physical and chemical properties, in line with carbon sequestration and soil health improvement goals. Historically, the IBI was among the first to establish global guidelines, but the EBC has emerged as the benchmark standard in Europe, distinguishing itself with a highly rigorous and detailed approach. The EBC covers every aspect of production, from feedstock sourcing to the production process and the final product's characterization. Recently, the World Biochar Certificate (WBC) was introduced, representing a crucial evolution in this landscape. The WBC is designed to harmonize and standardize certification requirements globally, transcending regional specificities and offering a single certification recognized worldwide. Its main function is to simplify the international trade of biochar and its associated carbon credits, creating a "common language" among producers, buyers, and investors. Unlike local standards, the WBC facilitates access to global markets, ensuring that a product

[17] IPCC, "2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories - Appendix4: Method for Estimating the Change in Mineral Soil Organic Carbon Stocks from Biochar Amendments: Appendix 4 Method for Estimating the Change in Mineral Soil Organic Carbon Stocks from Biochar Amendments: Basis for Future Methodological Development," 2019.

certified in one country is recognized and reliable anywhere else in the world. This focus on harmonization is essential for scaling biochar production and use globally, unlocking its full potential as a climate change mitigation tool.
In the emerging biochar industry, product quality certifications ensure safety, fitness for purpose, and traceability of the biochar itself. These schemes assess feedstock eligibility, production practices, and product characteristics (e.g., contaminants, nutrients, H:C, PAHs), not carbon accounting. These product certifications (EBC/IBI/WBC) can be prerequisites for sale into certain applications or supply chains. They are distinct from carbon-credit methodologies, which quantify, monitor, and verify carbon removal. Product certification focuses on the material; carbon-credit certification focuses on the removal claim.

- **European Biochar Certificate (EBC)**

The European Biochar Certificate (EBC) has published, and frequently updates, the guidelines for a sustainable production of biochar[1], defined as "*a porous, carbonaceous material that is produced by pyrolysis of biomass and is applied in such a way that the contained carbon remains stored as a long-term C sink or replaces fossil carbon in industrial manufacturing. It is not made to be burnt for energy generation*.". The document presents 7 different certification classes with related quality standard required: Basic materials, Consumer materials, Urban, Agro, Agro-organic, Feed, Feed plus (Table 4). The biochar classification implies the admissibility of the product regarding applicable laws, regulations, and relevant industry standards.

Table 4: EBC certification classes for biochar..

EBC -Certification Class		EBC-FeedPlus	EBC-Feed	EBC-AgroOrganic	EBC-Agro	EBC-Urban	EBC-ConsumerMaterials	EBC-BasicMaterials
Elemental analysis	Declaration of Ctot, Corg, H, N, O, S, ash							
	H / Corg	< 0.4		< 0.7				
Physical parameters	Water content, dry matter (as received and @ < 3mm particle size), bulk density (DM), WHC, pH, salt content, electrical conductivity of the solid biochar							
TGA	Needs to be presented for the first production batch of a pyroylsis unit							
Nutrients	Declaration of N, P, K, Mg, Ca, Fe							
Heavy metals	Pb	10 g t^{-1} (88%DM)	10 g t^{-1} (88%DM)	45 g t^{-1} DM	120 g t^{-1} DM	120 g t^{-1} DM	120 g t^{-1} DM	declaration, no limit values for certification
	Cd	0.8 g t^{-1} (88% DM)	0.8 g t^{-1} (88% DM)	0.7 g t^{-1} DM	1,5 g t^{-1} DM	1,5 g t^{-1} DM	1,5 g t^{-1} DM	
	Cu	70 g t^{-1}DM	70 g t^{-1}DM	70 g t^{-1}DM	100 g t^{-1} DM	100 g t^{-1} DM	100 g t^{-1} DM	
	Ni	25 g t^{-1} DM	25 g t^{-1} DM	25 g t^{-1} DM	50 g t^{-1} DM	50 g t^{-1} DM	50 g t^{-1} DM	
	Hg	0.1 g t^{-1} (88% DM)	0.1 g t^{-1} (88% DM)	0.4 g t^{-1} DM	1 g t^{-1} DM	1 g t^{-1} DM	1 g t^{-1} DM	
	Zn	200 g t^{-1} DM	200 g t^{-1} DM	200 g t^{-1} DM	400 g t^{-1} DM	400 g t^{-1} DM	400 g t^{-1} DM	
	Cr	70 g t^{-1} DM	70 g t^{-1} DM	70 g t^{-1} DM	90 g t^{-1} DM	90 g t^{-1} DM	90 g t^{-1} DM	
	As	2 g t^{-1} (88% DM)	2 g t^{-1} (88% DM)	13 g t^{-1} DM	13 g t^{-1} DM	13 g t^{-1} DM	13 g t^{-1} DM	
Organic contaminents	16 EPA PAH	6±2.4 g t^{-1} DM	CSI-declaration	6±2.4 g t^{-1} DM	6.0+2.4 g t^{-1} DM	CSI-declaration	CSI-declaration	CSI-declaration
	8 EFSA PAH	1.0 g t^{-1} DM						4 g t^{-1} DM
	benzo[e]pyrene benzo[j]fluoranthene	< 1.0 g t^{-1} DM for each of both substances						
	PCB, PCDD/F	See chapter 10		Once per pyrolysis unit for the first production batch. For PCB: 0.2 mg kg^{-1} DM, for PCDD/F: 20 ng kg^{-1} (I-TEQ OMS), respectively				

Table 5 shows how Juniperus communis biochar meets all the registration requirements of the EBC classes, especially those requiring high quality product characteristics: EBC-Agro, EBC-AgroOrganic and EBC-FeedPlus.

Table 5: Biochar compliance with EBC-Agro, EBC-Agro-Organic and Feed-Plus classes.

Parameter	UoM	EBC Thresholds	
		Agro	Agro Organic
H:C molar ratio	-	< 0.7	< 0.7
Cadmium (Cd)	mg/Kg db	< 1.5	< 0.7
Chromium (Cr)	mg/Kg db	< 90	< 70
Mercury (Hg)	mg/Kg db	< 1.0	< 0.4
Nickel (Ni)	mg/Kg db	< 50	< 25
Lead (Pb)	mg/Kg db	< 120	< 45
Arsenic (As)	mg/Kg db	< 13	< 13
Copper (Cu)	mg/Kg db	< 100	< 70
Zinc (Zn)	mg/Kg db	< 400	< 200
16 EPA PAH	mg/Kg db	< 6	< 6
8 EFSA PAH	mg/Kg db	< 1.0	< 1.0
Benzo(e)pyrene	mg/Kg db	< 1.0	< 1.0
Benzo(j)fluoranthene	mg/Kg db	< 1.0	< 1.0
PCB	mg/Kg db	< 0.2	< 0.2
PCDD/F	ng WHO tox. eq./Kg db	20.0	20.0

* chapter 10 of the guidelines[13]

1.5 Carbon removal

As global climate governance frameworks and consumer expectations increasingly prioritize emission reduction and carbon removal, initiatives aimed at mitigating Greenhouse Gas (GHG) concentrations are gaining economic relevance. The voluntary carbon market plays a critical role in mobilizing finance for such initiatives by providing mechanisms to quantify, verify, and monetize carbon sequestration efforts through scientifically validated approaches. Biochar has emerged as a highly effective and scalable carbon removal technology. In 2023, biochar-based projects accounted for approximately 94% of all long-duration carbon removal credits issued, underscoring their growing significance in achieving climate targets[18].

Biochar projects determine the volume of carbon sequestered using standardized, peer-reviewed methodologies. The removed carbon is quantified in metric tons of carbon dioxide equivalents (tCO_2e), ensuring consistency, comparability, and transparency in reporting. Upon successful third-party verification, these quantities are formally registered and issued as carbon credits—also referred to as removal credits—tradable within the carbon market. One metric ton of sequestered CO_2e corresponds to one verified carbon credit. In this system, project developers (sellers) represent certified carbon removal activities, while buyers are typically entities seeking to offset residual emissions that cannot be mitigated through direct reductions.

[18] www.cdr.fyi

1.5.1 Certification and Methodological Frameworks for Biochar

- **Voluntary credits market**

To participate in carbon markets, biochar producers must adhere to internationally recognized certification standards and approved methodologies that govern the quantification, monitoring, and verification of carbon removals. These standards are designed to ensure environmental integrity and a net positive climate impact.
The *Manual for Biochar Carbon Removal: A Comparative Guide for the Certification of Biochar Production as a Carbon Sink*, published by the International Biochar Initiative[19], identifies the leading certification programs currently active in the market:

- **Puro Standard**: The first certification body to develop and implement a methodology for biochar-based carbon removal. Puro oversees a wide portfolio of accredited projects using a scientifically rigorous sequestration model.
- **Carbon Standards International (CSI)**: Initially known for the European Biochar Certificate (EBC), CSI now offers:
    - *Global Biochar C-Sink Methodology*, applicable to a wide variety of biochar systems.
    - *Global Artisan C-Sink Methodology*, tailored for small-scale producers, including those in low- and middle-income regions.
- **Verified Carbon Standard (VCS)**: Administered by Verra, one of the most widely adopted carbon certification bodies. VCS's *VM0044 Methodology for Biochar Utilization* applies to both soil and non-soil uses of biochar globally.
- **Climate Action Reserve (CAR)**: A North America–focused program that has developed the *U.S. and Canada Biochar Protocol*, ensuring compliance with strict sustainability and carbon permanence criteria.
- **Riverse**: A European certification platform specializing in industrial-scale bio-based technologies. Its *BECCS & Biochar Methodology* certifies both biochar and bio-oil production systems.

These certification standards provide biochar producers with robust tools for validating carbon sequestration claims and accessing carbon finance through the issuance of verified credits. In parallel, biochar quality specifications are enforced to ensure environmental safety and appropriate end-use applications. Importantly, certified biochar is prohibited from being used as a fuel or reductant, as combustion would release the sequestered carbon, nullifying its climate mitigation potential. Furthermore, for a biochar project to qualify for removal credits, it must demonstrate carbon stability with a minimum permanence of 100 years. Only biochar that meets this durability threshold is eligible for credit issuance, ensuring that such projects contribute to long-term climate mitigation objectives.

- **CRCF – Carbon removal and carbon farming EU regulation**

Regulation (EU) No. 2024/3012[20], widely known as the Carbon Removal and Carbon Farming (CRCF), which was published in December 2024, establishes the framework for the voluntary certification of carbon removal credits within the European Union. This voluntary credit system is distinct from the EU's existing mandatory market, the Emissions Trading System (ETS), which sets the reduction

[19] www.biochar-international.org
[20] Regulation (EU) 2024/3012 of the European Parliament and of the Council of 27 November 2024 establishing a Union certification framework for permanent carbon removals, carbon farming and carbon storage in products.

targets for obligated entities (those required to offset their emissions). The CRCF Regulation introduces a unified certification scheme based on primary categories of carbon removal and emission reduction activities: Permanent Carbon Removals (PCRs), Carbon Storage in Long-lasting Products, and Carbon Farming. Within the CRCF, there is a key distinction between temporary and permanent storage. Biochar Carbon Removal (BCR) is anticipated to be included in the category of Permanent Carbon Removals, a designation that underscores its potential for long-term climate mitigation. The EU CRCF defines Permanent Carbon Removal as human activities removing CO_2 from the atmosphere and storing it securely and durably for several centuries. This stringent requirement for durability is what sets the CRCF apart and necessitates a reliable method for measuring the permanence of the stored carbon. Only the long-term durable carbon fraction of the biochar can be accounted for as permanent CDR under the CRCF specifications. Permanent storage may be achieved through the application of biochar to soils or by its incorporation into materials, subject to the detailed requirements within the CRCF specifications. The quantification of CO_2 removals and associated GHG emissions for biochar application involves precise rules and requirements. This information has been formalized in March 2025 within the "draft technical specifications for the certification of permanent carbon removals through biochar"[21]. These specifications contain all the detailed information necessary for accurately accounting for the climate benefits derived from the use of biochar. One method for assessing the permanence fraction of biochar is the direct assessment of the inertinite fraction within the material[22]. The other method to predict the long-term stability of the sequestered carbon is the H/Corg molar ratio. A low ratio (the CRCF limit is a maximum of 0.7) signifies higher aromaticity and thermal maturity. Operators using this option for permanence assessment must use the H/Corg ratio for the biochar and the expected average temperature for the location of biochar application/incorporation to calculate Fperm, which represents the permanence fraction of the biochar.

[21] Support to the development of methodologies for the certification of industrial carbon removals with permanent storage. Draft technical specifications for the certification of permanent carbon removals through biochar. ICF S.A., Cerulogy and Fraunhofer ISI. March 2025

[22] Sanei et al., "Assessing biochar's permanence: An inertinite benchmark" 2024. International Journal of Coal Geology 281, 104409

2. Lab scale carbonization

RE-CORD produced biochar from both lignocellulosic biomass (WP3) and bagasse materials (Task 4.1) to assess its potential agronomic application as soil improver. RE-CORD has collected different lignocellulosic biomass samples and bagasse material from respectively WP3 and WP4.

➔ **In the following table there is the list of the samples converted into biochar through lab scale carbonization.**

Table 6: Raw materials requirements for the manufacture of biochar.

Feedstock	Supplied by
Guayule bagasse	GUATECS (WP4)
Crambe	UNIBO (WP3)
Crambe, Galactica	HOHENHEIM (WP3)
Miscanthus, MxG	HOHENHEIM (WP3)
Yellow melilot	HOHENHEIM (WP3)
Common tansy	HOHENHEIM (WP3)
Hemp, Futura 83	HOHENHEIM (WP3)
Safflower hulls	CREA (WP3)
Safflower straw	CREA (WP3)

The feedstock received from WP3 and WP4 were characterized and then converted into biochar through lab scale carbonization at 550°C of maximum temperature, with a heating rate of 15 °C/min and a plateau at the maximum temperature of 40 minutes.
Biochar products were characterized in terms of proximate and ultimate analysis (moisture, volatile matter, fixed carbon, ash, C-H-N-S, micro- and macro-elements content). Furthermore, the H:C molar ratio will be evaluated to investigate the product stability (C-Sink potential of the biochar). Specific surface area (BET) and volume (DFT) were investigated as well.

2.1 Feedstock for biochar production at lab scale

Biochar samples were produced by slow pyrolysis in a macro TGA (LECO TGA 701, Figure 1A) under nitrogen flow. This instrument is equipped with 19 ceramic crucibles (Figure 1B) (volume 20 mL) with a sensitivity of mass measurement up to 0.1 mg. The equipment allows temperatures up to 1000 °C and, thanks to the rotation of the carousel, samples have uniform exposure to the process parameters set for the production test.
The thermal processes were performed with parameters listed in Table 7. The aim was to simulate the process parameters suitable for replication in RE-CORD pilot plant, where the heating rate and the residence time at the maximum temperature are correlated.

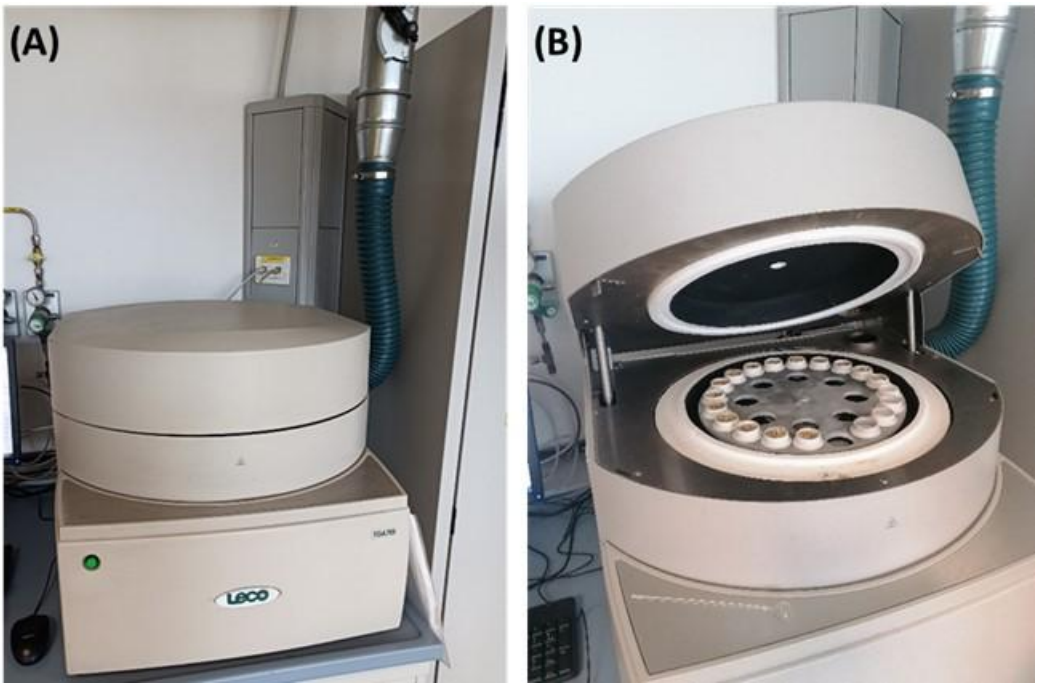


Figure 1: LECO TGA 701 at RE-CORD lab (A) and crucibles loaded with biomass samples (B).

Table 7: Slow pyrolysis parameter set for lab scale biochar production.

Parameter	Value	U.M.
Nitrogen flow	10	L min^{-1}
Heating Rate	20	°C min^{-1}
Maximum temperature	550	°C
Thermal plateau	40	min

- **Guayule bagasse by GUATECS**

Guayule bagasse was supplied by GUATECS. Biochar mass yield resulted 26.9 % wb

Figure 2: Guayule bagasse supplied by GUATECS.

Table 8: Guayule bagasse characterization.

Parameter	Value	U.M.
Internal code	RES.23.055.001	
Typology	Bagasse	
Crop	Guayule	
Moisture	12.8	%wt ar
Volatile matter	77.2	%wt db
Ash content	3.4	%wt db
C	51.9	%wt db
H	7.6	%wt db
N	1.3	%wt db
S	0.2	%wt db
O	35.6	%wt db
C d.a.f.	53.7	%wt db

- **Crambe biomass by UNIBO**

Crambe biomass was supplied by UNIBO. Biochar mass yield resulted 26.7 % wb

Figure 3: Crambe biomass as received (left) and milled (right).

Table 9: Crambe biomass characterization.

Parameter	Value	U.M.
Internal code	RES.23.055.007	
Typology	Lignocellulosic residue	
Crop	Crambe	
Moisture	8.0	%wt ar
Ash content	6.2	%wt db
Total C	46.9	%wt db
Total H	6.4	%wt db
Total N	0.4	%wt db
Total S	0.2	%wt db
O	39.9	%wt db
C d.a.f.	50.0	%wt db

- **Biomasses by Hohenheim University**

Multiple biomass samples were supplied by Hohenheim University. The sample are listed in Table 10 and shown in Figure 4.

Table 10: biomass samples supplied by Hohenheim University.

Sample code	Botanical name	Common name, Variety	Biomass type	Origin/ Site	Harvest date
RES.24.055.001	Crambe abyssinica	Crambe, Galactica	Straw (≈ stem)	OLI	05/09/23
RES.24.055.002	Miscanthus x giganteus	Miscanthus, MxG	Whole crop	WPM14	27/02/23
RES.24.055.003	Melilotus officinalis	Yellow melilot, n.a.	Whole crop	OLI	18/04/23
RES.24.055.005	Cannabis sativa	Hemp, Futura 83	Stem	OLI	29/09/23

Figure 4: Biomass samples as received by Hohenheim University.

Table 11: Feedstock characterization, Hohenheim's samples.

Crop	Crambe	Miscanthus	Yellow melilot	Hemp
Variety	Galactica	MxG	n.a.	Futura 83
Code	RES.24.055.001	RES.24.055.002	RES.24.055.003	RES.24.055.005
Moisture (%wt ar)	9.1	7.4	8.5	8.0
Volatile matter (%wt db)	75.0	79.6	79.1	77.9
Ash content (%wt db)	8.5	2.4	2.0	3.2
C (%wt db)	41.6	45.0	45.4	43.1
H (%wt db)	6.0	6.2	6.3	6.3
N (%wt db)	1.3	0.2	0.9	0.3
S (%wt db)	0.14	0.06	0.08	0.07

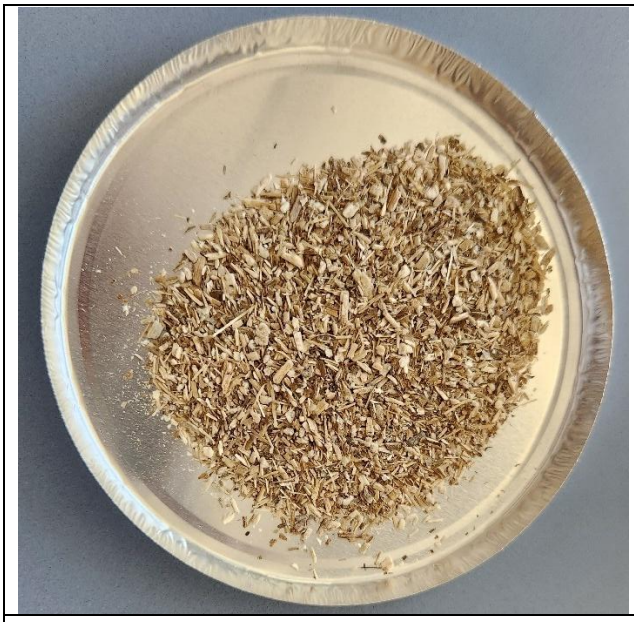
In *Table 12* are presented all the mass yield obtained with these sample during the thermochemical conversion through slow pyrolysis.

Table 12: process yields, Hohenheim's samples.

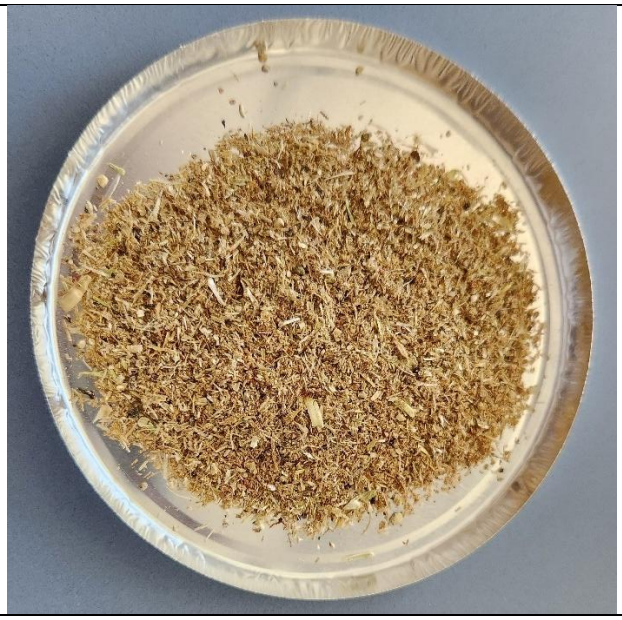
Feedstock code	Common name, Variety	Biochar code	Mass yield (%wt, wb)
RES.24.055.001	Crambe, Galactica	RES.24.055.010	27.5
RES.24.055.002	Miscanthus, MxG	RES.24.055.011	23.1
RES.24.055.003	Yellow melilot, n.a.	RES.24.055.012	24.2
RES.24.055.005	Hemp, Futura 83	RES.24.055.014	23.8

- **Safflower residues by CREA**

Safflower biomass residues (straw and hulls) were supplied by CREA. Biochar mass yield resulted respectively 25.2 and 28.7 % wb.



RES.24.055.019 - Safflower straw



RES.24.055.020 – Safflower hull

Figure 5: Safflower biomass samples supplied by CREA-IT.

Table 13: Safflower biomass residues characterization.

Parameter	Value		U.M.
Internal code	RES.24.055.019	RES.24.055.020	
Typology	Lignocellulosic residue		
Crop	Safflower		
Residue	Straw	Hull	
Moisture	9.2	7.9	%wt ar
Volatile matter	77.0	73.9	%wt db
Ash content	4.4	20.5	%wt db
Total C	47.8	45.4	%wt db
Total H	5.3	5.4	%wt db
Total N	0.2	1.9	%wt db
Total S	0.11	0.32	%wt db
O	46.5	46.9	%wt db
C d.a.f.	50.0	50.7	%wt db

2.2 Biochar characterization

All the biochar produced at lab scale were characterized following standards presented in Chapter 3.2, where biochar is intended for agronomic purposes.

2.2.1 Guayule biochar

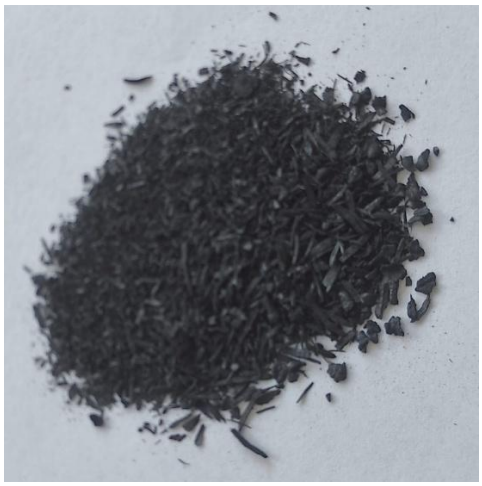


Figure 6: Guayule bagasse biochar.

Table 14: Guayule biochar characterization.

Parameter	Value	U.M.
Internal code	RES.23.055.002	
Feedstock typology	Bagasse	
Crop	Guayule	
Moisture	2.4	%wt ar
Volatile matter	14.8	%wt db
Ash content	12.4	%wt db
Fixed carbon	72.8	%wt db
Total C	79.1	%wt db
Total H	2.6	%wt db
Total N	1.9	%wt db
Total S	0.2	%wt db
Total O	3.7	%wt db
C d.a.f.	90.3	%wt db
H:C molar ratio	0.39	mol/mol
Specific surface area (BET)	6	m^2/g
Specific pore volume (DFT)	0.006	cm^3/g
Al	375	mg/kg db
B	b.d.l.	mg/kg db
Ba	13	mg/kg db
Ca	13055	mg/kg db
Cd	b.d.l.	mg/kg db
Co	b.d.l.	mg/kg db
Cr	0,2	mg/kg db
Cu	16	mg/kg db

Fe	286	mg/kg db
K	16826	mg/kg db
Li	b.d.l.	mg/kg db
Mg	757	mg/kg db
Mn	22	mg/kg db
Mo	b.d.l.	mg/kg db
Na	4727	mg/kg db
Ni	b.d.l.	mg/kg db
P	2438	mg/kg db
Pb	3,4	mg/kg db
Si	1083	mg/kg db
Ti	13	mg/kg db
V	b.d.l.	mg/kg db
Zn	b.d.l.	mg/kg db

2.2.2 Crambe biochar

Figure 7: Crambe biochar.

Table 15: Crambe residue biochar characterization.

Parameter	**Value**	**U.M.**
Internal code	RES.23.055.008	
Feedstock typology	Lignocellulosic residue	
Crop	Crambe	
Moisture	1.1	%wt ar
Volatile matter	19.5	%wt db
Ash content	18.7	%wt db
Fixed carbon	61,8	%wt db
Total C	74.9	%wt db
Total H	1.9	%wt db
Total N	0.9	%wt db
Total S	0.08	%wt db
Total O	22.2	%wt db

C d.a.f.	92.2	%wt db
H:C molar ratio	0.30	mol/mol
Specific surface area (BET)	8	m^2/g
Specific pore volume (DFT)	0.010	cm^3/g
Al	88	mg/kg db
B	24	mg/kg db
Ba	61	mg/kg db
Ca	26584	mg/kg db
Cd	b.d.l.	mg/kg db
Co	b.d.l.	mg/kg db
Cr	b.d.l.	mg/kg db
Cu	b.d.l.	mg/kg db
Fe	83	mg/kg db
K	43687	mg/kg db
Li	b.d.l.	mg/kg db
Mg	1823	mg/kg db
Mn	30	mg/kg db
Mo	b.d.l.	mg/kg db
Na	215	mg/kg db
Ni	b.d.l.	mg/kg db
P	1654	mg/kg db
Pb	b.d.l.	mg/kg db
Si	b.d.l.	mg/kg db
Ti	b.d.l.	mg/kg db
V	b.d.l.	mg/kg db
Zn	b.d.l.	mg/kg db

2.2.3 Hohenheim's biomasses biochar

	
RES.24.055.010 Crambe biochar	RES.24.055.011 Miscanthus biochar

Picture not available: All the biochar sample was used for its characterization.	
RES.24.055.012 Yellow melilot biochar	RES.24.055.014 Hemp biochar

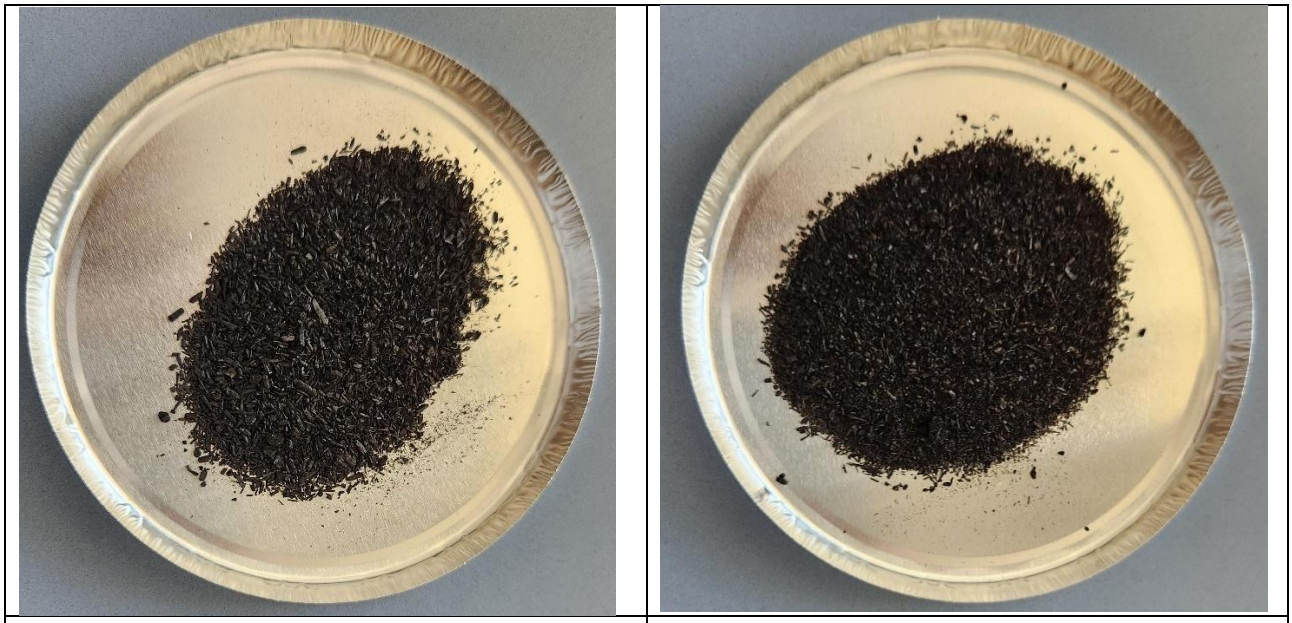
Figure 8: Biochar samples produced with the biomasses received by Hohenheim University.

Table 16: Biochar characterization, Hohenheim's samples.

Internal code		**RES.24.055.010**	**RES.24.055.011**	**RES.24.055.012**	**RES.24.055.014**
Feedstock typology		Lignocellulosic residue	Lignocellulosic residue	Lignocellulosic residue	Lignocellulosic residue
Crop		Crambe	Miscanthus	Yellow melilot	Hemp
Moisture	%wt ar	4.1	4.6	4.8	5.9
Volatile matter	%wt db	21.2	14.0	17.8	16.9
Ash content	%wt db	25.8	9.6	7.5	12.4
Fixed carbon	%wt db	54.9	77.4	75.8	70.6
Total C	%wt db	66.7	82.0	81.2	79.9
Total H	%wt db	1.6	2.1	2.2	1.8
Total N	%wt db	1.8	0.5	2.3	0.7
Total S	%wt db	0.14	0.07	0.07	0.03
Total O	%wt db	29.7	15.3	14.3	17.6
C d.a.f.	%wt db	88.7	90.3	85.3	91.3
H:C molar ratio	mol/mol	0.28	0.30	0.32	0.27
Specific surface area (BET)	m^2/g	27	195	172	36
Specific pore volume (DFT)	cm^3/g	0.027	0.117	0.107	0.029
Al	mg/kg db	267	207	767	277
B	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Ba	mg/kg db	0.7	33	0.7	5
Ca	mg/kg db	46964	6012	15091	18446
Cd	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Co	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Cr	mg/kg db	0.9	5	3	5
Cu	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Fe	mg/kg db	169	141	442	180

K	mg/kg db	35195	11075	3543	29040
Li	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Mg	mg/kg db	1581	1363	924	2085
Mn	mg/kg db	46	181	59	80
Mo	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Na	mg/kg db	137	142	15	31
Ni	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
P	mg/kg db	6816	1008	1424	1985
Pb	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Si	mg/kg db	480	2159	1089	626
Ti	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
V	mg/kg db	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Zn	mg/kg db	b.d.l.	33	23	b.d.l.

2.2.4 Safflower residues biochar



RES.25.055.001 - Safflower straw biochar | RES.25.055.002 – Safflower hull biochar

Figure 9: Biochar of the safflower biomass samples supplied by CREA-IT.

Table 17: Safflower biomass residues biochar characterization.

Parameter	Value		U.M.
Internal code	RES.25.055.001	RES.25.055.002	
Feedstock typology	Lignocellulosic residue		
Crop	Safflower		
Residue	Straw	Hull	
Moisture	1.9	2.4	%wt ar
Volatile matter	16.3	20.6	%wt db
Ash content	15.0	31.5	%wt db
Fixed carbon	68.8	47.9	%wt db
Total C	79.4	61.8	%wt db
Total H	2.2	1.7	%wt db
Total N	0.5	2.5	%wt db

Total S	0.07	0.40	%wt db
Total O	17.8	33.6	%wt db
C d.a.f.	93.4	90.2	%wt db
H:C molar ratio	0.33	0.34	mol/mol
Specific surface area (BET)	47	9	m^2/g
Specific pore volume (DFT)	0.038	0.011	cm^3/g
Al	1228	213	mg/kg db
B	b.d.l.	37	mg/kg db
Ba	204,7	63,1	mg/kg db
Ca	33746	49811	mg/kg db
Cd	b.d.l.	b.d.l.	mg/kg db
Co	b.d.l.	b.d.l.	mg/kg db
Cr	b.d.l.	b.d.l.	mg/kg db
Cu	6	41	mg/kg db
Fe	613	227	mg/kg db
K	19585	54050	mg/kg db
Li	1	0.3	mg/kg db
Mg	2002	6251	mg/kg db
Mn	44	38	mg/kg db
Mo	b.d.l.	b.d.l.	mg/kg db
Na	663	617	mg/kg db
Ni	b.d.l.	b.d.l.	mg/kg db
P	2111	5145	mg/kg db
Pb	b.d.l.	b.d.l.	mg/kg db
Si	4664	580	mg/kg db
Ti	60	b.d.l.	mg/kg db
V	b.d.l.	b.d.l.	mg/kg db
Zn	54	219	mg/kg db

2.3 Conclusions

2.3.1 Product quality evaluation

Biochar's main parameters for the quality standard of the material for agronomic application as soil improver are listed in Table 3, Table 4 and Table 5.
Salmonella spp. and Escherichia coli (or Enterococcaceae) are used as hygienic (or sanitation) indicators, thus in a lab scale evaluation are not considered, because are relevant in an industrial scale environment.
Polycyclic Aromatic Hydrocarbons (PAH16), Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (PCDD/F), and non-dioxin-like Polychlorinated Biphenyls (PCB) are organic pollutants whose formation is highly dependent on the plant design and operating conditions; consequently, they were not analysed during the laboratory-scale production phase.
The focus shifted to the analysis of the material's fundamental properties, specifically the heavy metal content, carbon content, and the H/C molar ratio. These analyses were supplemented with measurements of the specific surface area and specific pore volume to highlight any development of micro- and meso- porosity in the material. These values, in turn, provide key insights into the material's potential applications, such as nutrient and water retention.

The organic carbon content resulted well higher than the threshold given by the EU FPR because the regulation's minimal limit is set extremely low to accommodate a wide variety of inorganic or minimally processed fertilizing material categories, reflecting the EU's broader effort to integrate diverse recycled and biogenic materials into the circular economy. The highest ash content resulted from the Safflower hulls, reaching 31.5% by weight on a dry basis. The Fixed Carbon content of the materials produced directly reflects their total carbon and ash content. Furthermore, evaluation of the material's stability using the H/C molar ratio demonstrates high stability, with the value always below 0.4 and remaining around 0.3 in most cases.

Table 18: Re-cap of carbon contents of the samples produced.

Parameter	Guayule	Crambe	Crambe	Miscant.	Y.Melilot	Hemp	Saffl._1	Saffl._2
	GUATECS	UNIBO	HOHEN.	HOHEN.	HOHEN.	HOHEN.	CREA	CREA
Total C (%wt db)	79.1	74.9	66.7	82.0	81.2	79.9	79.4	61.8
Fixed C (%wt db)	72.8	61.8	54.9	77.4	75.8	70.6	68.8	47.9
Ashes (%wt db)	12.4	18.7	25.8	9.6	7.5	12.4	15.0	31.5
H/C molar ratio	0.39	0.30	0.28	0.30	0.32	0.27	0.33	0.34

In Table 19 the Specific Surface Area (SSA) and Specific Prove Volume (DFT) are listed for a direct comparison. Biochars with a higher specific surface area and a higher potential water and nutrient retention are the ones produced from *Miscanthus (MxG)* and Yellow Melilot. All the others resulted in very poor development of micro- and meso-porosity.

Table 19: Re-cap of specific surface area and pore volume of the samples produced.

Parameter	Guayule	Crambe	Crambe	Miscant.	Y.Melilot	Hemp	Saffl._1	Saffl._2
	GUATECS	UNIBO	HOHEN.	HOHEN.	HOHEN.	HOHEN.	CREA	CREA
SSA (m^2/g)	6	8	27	195	172	36	47	9
SPV (cm3/g)	0.006	0.010	0.027	0.117	0.107	0.029	0.038	0.011

In Table 21 all the results about heavy metals analyzed for the biochars produced are listed for a direct comparison with the regulation and quality standard threshold. Table 20 lists the specific thresholds for both EU FPR and EBC. Mercury (Hg) and Arsenic (As) contents were not investigated.

Table 20: Re-cap of heavy metals thresholds for EU FPR and EBC.

Parameter	**EU FPR PFC 3(A)** ($mg\ kg^{-1}$ db)	**EBC Agro** ($mg\ kg^{-1}$ db)	**EBC Agro organic** ($mg\ kg^{-1}$ db)
Cadmium	< 2.0	< 1.5	< 0.7
Chromium	< 2.0*	< 90	< 70
Mercury	< 1.0	< 1.0	< 0.4
Nickel	< 50	< 50	< 25
Lead	< 120	< 120	< 45
Arsenic	< 40**	< 13	< 13
Copper	< 300	< 100	< 70
Zinc	< 800	< 400	< 200

*Hexavalent Chromium (Cr VI)

**Inorganic Arsenic

Table 21: Comparison of heavy metals analysed in the biochar samples produced.

Parameter (mg kg^{-1} db)	Guayule GUATECS	Crambe UNIBO	Crambe HOHEN.	Miscant. HOHEN.	Y.Melilot HOHEN.	Hemp HOHEN.	Saffl._1 CREA	Saffl._2 CREA
Cadmium	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Chromium	0.2	b.d.l.	0.9	5	3	5	b.d.l.	b.d.l.
Nickel	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Lead	3.4	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Copper	16	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	6	41
Zinc	b.d.l.	b.d.l.	b.d.l.	33	23	b.d.l.	54	219

All the samples produced resulted with no particular issues related to heavy metal contamination. **Specifically, guayule bagasse, the two crambe biomasses, miscanthus, yellow melilot, hemp and safflower straw, all demonstrated excellent biochar characteristics based on the analyses.**
A contamination issues regarding the Zinc (Zn) content in the Safflower hulls would exclude its use in organic agriculture according to the limit imposed by the EBC (European Biochar Certificate), although the exceedance was minimal.

3. Biochar production for agronomic trials

Poplar wood chips were used as feedstock to produce the biochar for the agronomic on field trial (Figure 10). More than 120 kg of biochar were produced at demo scale and full characterized to check the compliance with EU Fertilizing Product Regulation 1009/2019, as CMC 14 (biochar) and PFC 3A (soil improver). The biochar was then shipped to the partners involved in the agronomic trials.

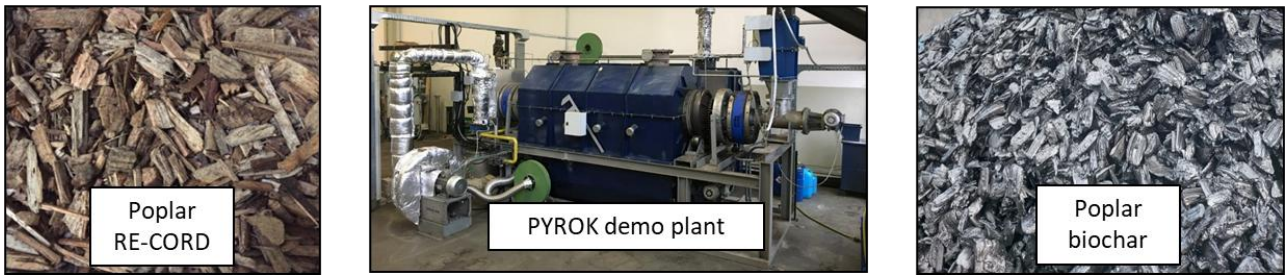


Figure 10: Schematic representation of the carbonization at demo scale

3.1 Biochar production at demo scale

The pyrolysis plant used for the pilot scale test, named PYROK, is a rotary drum pyrolysis reactor owned by RE-CORD (Figure 1). The plant can process up to 100 kg/h of raw material, and consists of a main pyrolysis section, where the rotary drum is located inside a muffle in which the produced pyrolysis gas is burned.

Figure 11: "PYROK" pyrolysis unit with vapor condensation system for sampling.

The reactor is maintained at a slightly negative pressure using a high-temperature fan that allows the extraction of pyrogas, while the biochar is recovered by an auger conveyor. The generated pyrogas is first sent to a cyclone, to remove the particulate matter present in the gas, and then it can be combusted in two combustion chambers: a primary one (muffle), intended for supplying the necessary heat to the reactor, and a secondary one, for disposing of excess heat. Both chambers are equipped with a dual fuel burner, fed not only by pyrogas but also by natural gas. The combustion fumes are then cooled by a water heat exchange system before being sent to the chimney.

The feedstock used was poplar wood chips, as shown in Figure 12.

Figure 12: Poplar wood chips.

30 kg of poplar biochar, on a dry basis, (as shown in Figure 13) were shipped to UNICT, NOVAMONT, CRES and CIEMAT. A technical datasheet with biochar full characterization was sent to the partners. The document summarizes the information included in this chapter (Figure 13).

Figure 13: Poplar wood biochar ready to be shipped (left), biochar technical datasheet (right).

3.2 Biochar characterization for agronomic purpose

Main physico-chemical characteristics, metals and organic contaminants concentrations of the poplar biochar (Figure 14) produced in the RE-CORD Pyrok plant through slow pyrolysis process (T_{MAX}= 550°C), are shown in Table 23. Parameters and related analytical methods are shown in the following Table 22.

Table 22: Parameters and related analytical methods.

Parameter	Analytic method
Moisture	UNI EN ISO 18134-2
Volatile matter	UNI EN ISO 18122
Ash content	UNI EN ISO 18123
Fixed carbon	Calculated
Total C, H, N	UNI EN ISO 16948
Inorganic C	D.M. 13/09/99 Met. V.1
Organic C	Calculated
H:C molar ratio	Calculated
pH	ISO 10390
Bulk density	UNI EN ISO 17828
EC (1:10)	ISO 11265
WHC (% m/m)	DM 01/08/1997 Met. 5
Germination index	UNI 10780:1998 App.K
Specific surface area (BET)	ASTM D6556-10
Specific pore volume (DFT)	ASTM D6556-10
Granulometry	UNI EN ISO 17827-1
Micro and macro-elements: Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Si, Ti, V, Zn	UNI EN ISO 16967 + UNI EN ISO 16968
As, Tl, Hg	UNI EN 13657:2004 + UNI EN ISO 11885:2009
Cr VI	DM 08/05/2003
PAHs	UNI EN 16181
Sum PCDDs+PCDFs	EPA 31613B 1994
PCBs	EPA 1668 C 2010

Figure 14: Poplar biochar produced in RE-CORD demo-plant.

The material characterization was done following the European Fertilizing Product Regulation (EU Reg. 1009/2019, considering the parameters related to CMC14 (biochar) and PFC3(A) (soil improver). Specific requirements for CMC 14 (biochar) refer to organic pollutants content, such as PAH-16 (polycyclic aromatic hydrocarbons), PCDD/F (dioxins and furans) and PCB (polychlorinated biphenyls. CMC 14 can be used alone or combined with other CMCs to constitute a PFC. The Product Function Categories n.3A and n.4, for example, permit to characterise a product as soil improver (PFC 3A) or growing medium (PFC 4). These classifications included thresholds for heavy metals content, such as Cd (Cadmium), Cr VI (Hexavalent Chromium), Hg (Mercury), Ni (Nickel), Pb (Lead), As (Inorganic Arsenic), Cu (Copper) and Zn (Zinc). In conclusion, biochar that complies the EU FPR requirements, is a very clean and safe vegetable carbon to be used for agronomic purposes.

Table 23: Biochar characterization.

Parameter	Value	U.M.
Feedstock typology	Wood chips	
Specie	Poplar	
Moisture	2.3	%wt ar
Volatile matter	8.0	%wt db
Ash content	11.7	%wt db
Fixed carbon	80.4	%wt db
Total C	86.92	%wt db
Total H	2.07	%wt db
Total N	0.29	%wt db
Inorganic C	0.48	%wt db
Organic C	86.4	%wt db
H:C molar ratio	0.28	mol/mol
pH	8.0	-
Bulk density	263	Kg/m^3 a.d.
EC (1:10)	4.35	mS/m
WHC (% m/m)	143	%wt
Germination index	57	%
Specific surface area (BET)	88	m^2/g
Specific pore volume (DFT)	0.055	cm^3/g
Granulometry		
< 0,5 mm	21.37	%wt
> 0,5 mm	6.19	%wt
> 1 mm	7.52	%wt
> 2 mm	16.85	%wt
> 5 mm	48.07	%wt
d50	1.89	%wt
Micro and Macro-elements		
Al	667.7	mg/kg db
B	b.d.l.	mg/kg db
Ba	45.3	mg/kg db
Ca	23141	mg/kg db
Cd	<0.20	mg/kg db
Co	b.d.l.	mg/kg db
Cr	6.7	mg/kg db

Cu	b.d.l.	mg/kg db
Fe	893.5	mg/kg db
K	4964	mg/kg db
Li	0.9	mg/kg db
Mg	2571	mg/kg db
Mn	74.0	mg/kg db
Mo	b.d.l.	mg/kg db
Na	321	mg/kg db
Ni	b.d.l.	mg/kg db
P	b.d.l.	mg/kg db
Pb	b.d.l.	mg/kg db
Si	1129	mg/kg db
Ti	32.6	mg/kg db
V	b.d.l.	mg/kg db
Zn	92.2	mg/kg db
As	<2	mg/kg db
Tl	<1	mg/kg db
Hg	<1	mg/kg db
Cr VI	<0.5	mg/kg db
Organic pollutants		
PAHs	<4	mg/kg db
Sum PCDDs+PCDFs	0.65	ngTE/kg db
PCBs	0.0151	mg/kg db

a.r.= as received
db= dry basis
b.d.l.= below detection limit

All the parameters confirm the compliance of the material as an organic soil improver for agronomic applications.

4. Other potential biochar applications, preliminary investigation

The wide array of applications for biochar in agriculture and environmental contexts is expanding rapidly. Given its plant-derived origin, and assuming its properties align with relevant legislation, biochar is a highly versatile material. Within this project, activities of preliminary investigation were dedicated to evaluating two different biochar applications based on its unique properties, such as its role in **film mulching** and **seed coating**.

4.1 Biochar for mulch films

The interest in integrating biochar, particularly of the fine particle size, into *mulch films* stems from its unique properties that can enhance the agronomic and environmental performance of the films themselves. Acting as a filler or additive, biochar offers multiple benefits: its porous and dark structure can potentially modify the film's thermal properties, influencing soil temperature and crop growth. Furthermore, in the context of increasing use of biodegradable films, the addition of biochar may help improve their mechanical properties and, critically, positively influence the biodegradation process in the soil once their function is complete. Finally, utilizing biochar derived from agricultural or forestry waste as an additive represents a circular economy approach, turning a byproduct into a value-added component for a plastic material used in agriculture.

The biochar produced for the agronomic field trial, was further processed (milled and sieved) to obtain a fine powder to be shipped to NOVAMONT for evaluating its potential use as filler for mulch films.

Figure 15: Schematic representation of the production chain of biochar with a particle size <40 µm.

With this purpose, 2 kg of poplar biochar, were milled and then sieved at 40 µm before to be shipped to NOVAMONT, as shown in Figure 15. A ball miller was used (Figure 16) to reduce the particle size of the biochar.

Figure 16: ball miller (left) and biochar ready to be shipped (right).

The material provided by RE-CORD lab was sieved to 40 µm and no finer (Figure 17). Novamnont will evaluate the feasibility of further reducing this particle size to ensure optimal compatibility and performance, allowing the biochar to be effectively utilized as a functional filler within the mulch film material.

Figure 17: biochar sieved at 40 µm.

The material, compliant with EU FPR 1009/2019 for agronomic use, resulted with a particle size distribution excessively large to be used as shipped. This necessitates a further reduction step, which couldrepresents a challenge regarding energy demand.

4.2 Biochar for seed coating

Another promising example of biochar employment is its use in seed coating technology, which represents a strategic approach to enhance crop establishment. Consistent with the MIDAS project's focus, the investigation of biochar-based seed coating is explored here as a strategy to boost productive performance in marginal or disadvantaged soils.
To further explore this potential development, preliminary investigation activities were conducted. These activities involved utilizing biochar and selected seeds of agronomic interest to establish the fundamental feasibility of the application. Additionally, a germination trial was executed within a controlled climatic chamber to preliminarily assess the biochar's potential stimulatory or inhibitory effect on seed development. The results presented here are strictly introductory, intended to frame the topic by delineating the initial opportunities and limitations associated with this specific biochar application.

- **Seed coating**

Achieving successful seed germination and seedling establishment are fundamental prerequisites for profitable crop production and effective nature restoration efforts. However, productivity is often limited by several factors. Many crops are cultivated in marginal, low-fertility soils under suboptimal conditions, leading to early-stage stress. Furthermore, specific species can present inherent difficulties, such as low seed vigor, seed aging, or natural seed hardness[23], all of which impede successful establishment. After planting, seeds and seedlings are subjected to a range of abiotic and biotic stress factors, including pests, diseases, and, critically, moisture stress, especially common in dryland farming during the initial growth phases. These stresses can delay, reduce, or even compromise stand establishment and ultimately impact yield. In environmental contexts, direct seeding is a prevalent restoration practice[24]. Yet, its success is frequently hampered by poor germination rates and the challenge of competing with invasive species.

In this context, innovative strategies are essential to mitigate these risks and improve plant performance. Seed enhancement technologies, such as seed coating, offer a powerful solution to overcome these challenges. Seed coating is a reliable technique for applying exogenous materials in close proximity to the germinating seed, which effectively boosts seed quality and yield[25]. By enhancing seed placement and performance, it is particularly useful in situations where water and nutrient availability are limited. Materials used in seed coatings can be broadly categorized according to their function: binders, fillers, and active ingredients. Binders and fillers, provides the necessary thermal, physical, and mechanical characteristics to the coated seed, such as improved flowability and reduced seed skip. These components also serve to alter the original shape, size, and weight of the seeds, which significantly facilitates handling and sowing. It is crucial that binders and fillers are compatible with the active components and do not compromise the seed's ability to ger-

[23] Wang, et al., "*The effect of storage condition and duration on the deterioration of primed rice seeds*". 2018. https://doi.org/10.3389/fpls.2018.00172
[24] Williams et al., "*Can biochar be used as a seed coating to improve native plant germination and growth in arid conditions*?" 2016. http://dx.doi.org/10.1016/j.jaridenv.2015.09.011
[25] Zhang et al., "*Biochar coating is a sustainable and economical approach to promote seed coating technology, seed germination, plant performance, and soil health*" 2022. https://doi.org/10.3390/plants11212864

minate. Active ingredients, comprises substances specifically targeted to protect the seed and enhance germination and seedling development. Examples of these active agents include the application of nutrients, plant growth regulators, and beneficial microorganisms.

- **Biochar application seed coating material**

The growing interest in the specific physicochemical properties of biochar has led to its emergence as an innovative material for seed coating applications. This material can be effectively integrated into the coating matrix. Generally, biochar and seeds are mixed and blended together with an appropriate binding agent (e.g., starch) within a rotary tumbler until the required coating thickness is attained. This technique can be highly versatile and in literature it is possible to find several coating method approaches. Existing research has extensively explored the application of biochar as a seed coating material, typically utilizing biochar derived from herbaceous[26,27] or hardwood biomass[28,29]. Numerous studies have investigated biochar coating effects across various plant species under diverse conditions, ranging from laboratory settings[30,31] to field trials[32,33]. A common focus of this research has been the evaluation of seed coating efficacy in mitigating common environmental stresses, such as salinity[30] or water deficit [24]. However, the literature also reports different results and impacts. These discrepancies are often attributable to variations in the specific coating procedure used, the biochar and seed ratios applied, and the differing experimental conditions. Consequently, it is crucial to recognize that optimizing the coating protocol (considering the biochar dosage, the application technique, and the target species) is essential for achieving specific desired outcomes and maximizing the benefits of this technology.

- **Preliminary evaluation of biochar as seed coater**

This preliminary evaluation was conducted as part of the project with the primary goal of investigating the effect of biochar-based seed coating on three plant species characterized by distinct seed sizes and shapes: bean, maize, and sorghum.
The biochar was finely milled to achieve a very small particle size, specifically ranging from 20 to 75 micrometers. Polyethylene glycol (PEG) (Figure 18) was employed as the binder, tested at two different dilutions.

[26] Camara-Williams "*Effect of Seed Pelleting and Biochar on Nodulation, Growth and Yield of Soybean (Glycine max L.)*" Doctoral Dissertation, University of Ghana, Accra, Ghana, 2019
[27] Ghazi "*Potential for biochar as an alternate carrier to peat moss for the preparation of Rhizobia bio inoculum*" 2017, https://doi.org/10.9734/MRJI/2017/30828.
[28] Głodowska et al., "*Biochar is a growth-promoting alternative to peat moss for the inoculation of corn with a pseudomonad*" 2016. https://doi.org/10.1007/s13593-016-0356-z
[29] Brown et al., "*Carbon-based pelleting, soil ripping and herbicide application can be used to overcome plant recruitment barriers in Grey Stinkwood (Jacksonia furcellata)*" 2023. doi: 10.1111/emr.12583
[30] Thomas et al., "*Polyvinyl acetate binders undermine the effectiveness of biochar-based seed coatings*" 2024. https://doi.org/10.3390/land13070941
[31] Banu et al., "*Biochar coating and its effect on seed quality and biochemical parameters in black gram* (*Vigna mungo* L.) var. VBN 11" 2022.
[32] Accinielli et al., "*Use of a biochar-based formulation for coating corn seeds*" 2023. https://doi.org/10.1080/23311932.2023.2283956
[33] Zhang et al., "*Germination and growth performance of water-saving and drought-resistant rice enhanced by seed treatment with wood vinegar and biochar under dry direct-seeded system*" 2022. https://doi.org/10.339

Figure 18: Polyethylene glycol (PEG) used as binder material (left) and milled biochar (right), used for seed coating.

The coating procedure involved mixing a measured quantity of biochar, the binder solution, and the seeds. Following application, the coated seeds were left to air-dry for four days in a dark, dry environment before planting. Figure 19 illustrates each species before and after the coating: bean, maize and sorghum.

Figure 19: Bean seed (left), maize seed (centre), sorghum seeds (right); before and after coating with biochar (right).

Three types of coating were prepared:

- untreated seed (as control treatment);
- seeds coated with PEG and biochar;
- seeds coated with PEG, biochar, and added water (representing the second binder dilution).

Germination was carried out using sieved peat (pre-dried and sieved to 2 mm) as the growing medium, and tests were conducted in the climate chamber under controlled conditions: a constant ambient temperature of 25°C and a 12-hour light/12-hour dark cycle. The irrigation level was maintained at 75% of the substrate's field capacity, ensuring the trial was performed in the complete absence of water stress.

Results of this preliminary investigation showed species-specific responses. Sorghum emerged as the highest-performing species, with seeds coated with PG (in both dilution variations) achieving faster and higher total germination rates than the untreated control (Figure 20).

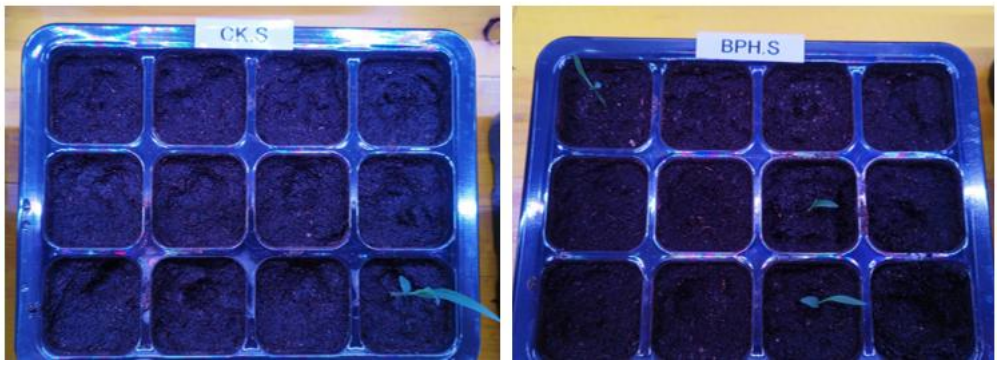


Figure 20. Control sorghum seed (left) and coated sorghum seed coated with biochar (right) during the germination trial.

Conversely, coated maize seeds (Figure 21) showed a neutral response, exhibiting no significant difference in germination compared to the control. In bean seeds, the coating resulted in a negative effect, causing lower germination rates than the control (Figure 22).

Figure 21: coated maize seeds germination in the tray.

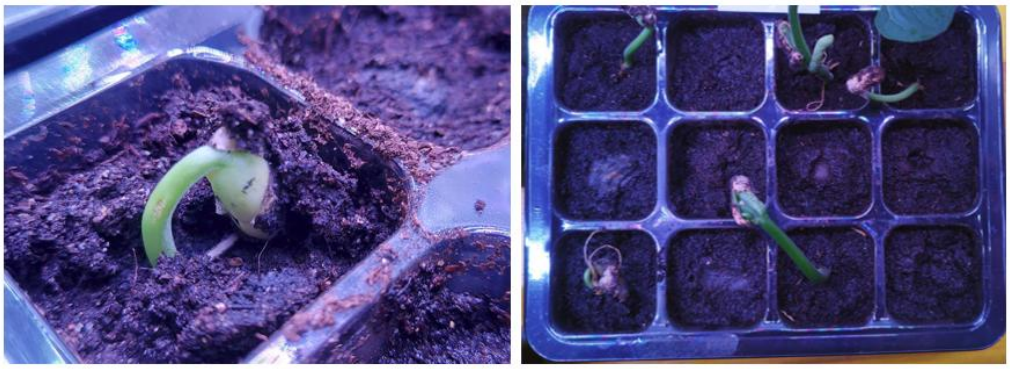


Figure 22: Coated bean seeds germination in the tray.

- **Results and lesson learnt**

These findings suggest a positive baseline effect for sorghum and maize under non-stress conditions, contrasting with the negative response of the bean. This discrepancy could suggest that for bean seeds, it may be necessary to either evaluate the coating under more challenging conditions (e.g., severe water stress) or, more importantly, optimize the coating protocol by testing alternative binders and different biochar typologies. The detrimental effect observed on the bean may be related to the PG product itself, reinforcing the need to use alternative, possibly biological, products like starch, which are less likely to negatively impact germination.

5. Wood vinegar production

The study's primary goal is the valorisation of lignocellulosic condensate, a co-product derived from slow pyrolysis, for potential agronomic applications. This valorisation capitalizes on the condensate's high acetic acid content as a source of biostimulant activity. The experimental methodology focuses on testing the efficacy of the diluted wood vinegar (the purified condensate) for pre-sowing seed imbibition. This approach aims to enhance seed germination rates and improve early seedling vigor, particularly under challenging water stress conditions. A simplified scheme is represented in Figure 23, where starting from the feedstock, the final product is presented.

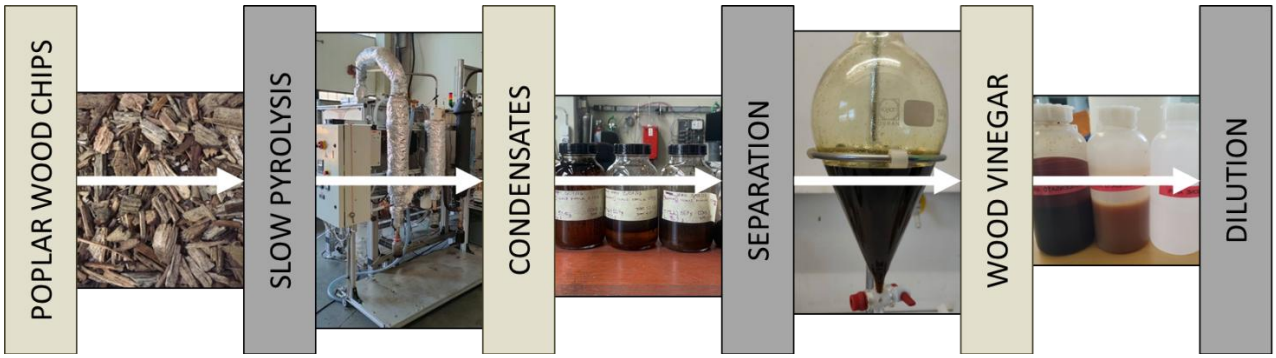


Figure 23: Simplified scheme representing wood vinegar production and dilution.

Poplar wood chips have been processed through slow pyrolysis process, obtaining as products both biochar and condensate. The details of the pilot-scale slow pyrolysis process and the subsequent liquid product recovery are extensively reported in the PhD thesis by G. Lombardi at RE-CORD[34]. Condensate is composed by three different fractions: light and heavy oil, plus the aqueous phase (Table 24).

Table 24: samples collected.

Samples	Code
Poplar	RES.22.046.044
Char	RES.23.046.091
Light oil phase	RES.23.046.097
Heavy oil phase	RES.23.046.098
Aqueous phase	RES.23.046.099

5.1 Plant description

Pilot scale production tests were performed in a continuous pyrolysis reactor (SPYRO) coupled with a condensation unit, Figure 24. SPYRO is a shaftless auger-type pyrolizer, whose reactor is approximately 2 m long and has an inner diameter of 0.15 m. The unit can convert up to 3 kg h^{-1} of biomass and can be operated up to 600°C.

[34] G. Lombardi, "Process Intensification of Biomass Slow Pyrolysis Through Autothermal Operation of Auger Reactor," Tesi di Dottorato, Univ. di Firenze, Firenze, Italia, 2024. [Online]. Disponibile su: https://hdl.handle.net/2158/1402032.

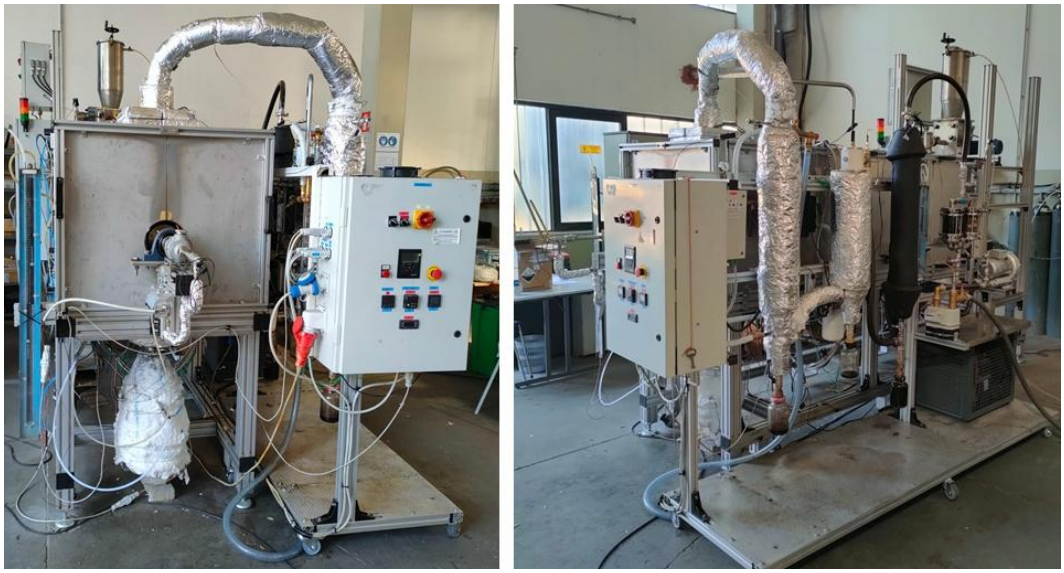


Figure 24: SPYRO pilot plant at RE-CORD experimental area (Scarperia, Florence, Italy).

With refers to Figure 25, solid biomass is introduced into the reactor by a dosing screw; the heat required for the pyrolysis process is provided by three radiative electric heaters, which are independently controlled and monitored via PLC in order to achieve the desired heating rate and to provide an extra degree of flexibility. The temperature feedback for each electric heater is provided through two K-type thermocouples, one monitoring the temperature of the reactor and the other between the heater and the reactor itself. Furthermore, two K-type thermocouples monitor the hot vapours temperature along the reactor. During the operation, nitrogen is adopted as purge gas and fed into the reactor to ensure an inert atmosphere. The rotating speed of the reactor screw can be adjusted to vary the solids residence time, which can range from few minutes up to 1 hour, so that the effect of the latter on the pyrolysis products and quality can be investigated. The charcoal is collected by gravity in a sealed vessel, while hot vapours are constantly withdrawn by a fan placed in the cold section, downstream of the condensation unit. Both outlets are heat-traced by independent conductive electric heaters to avoid organics condensation. Each heater is provided by a K-type thermocouple for temperature control and monitoring. A hot filter avoids excessive fouling in the condensation unit.
The latter unit consists in

- a tube-in-tube heat exchanger, named the hot condenser, operating up to 120 °C, in which the heavier fraction of the vapours is collected,
- an electrostatic precipitator (ESP),
- a shell-and-tube heat exchanger, named the cold condenser, operating around 0 °C, in which most of the aqueous phase is collected,
- a filtration section,
- a fan, which provides the suction for the pyrogas.

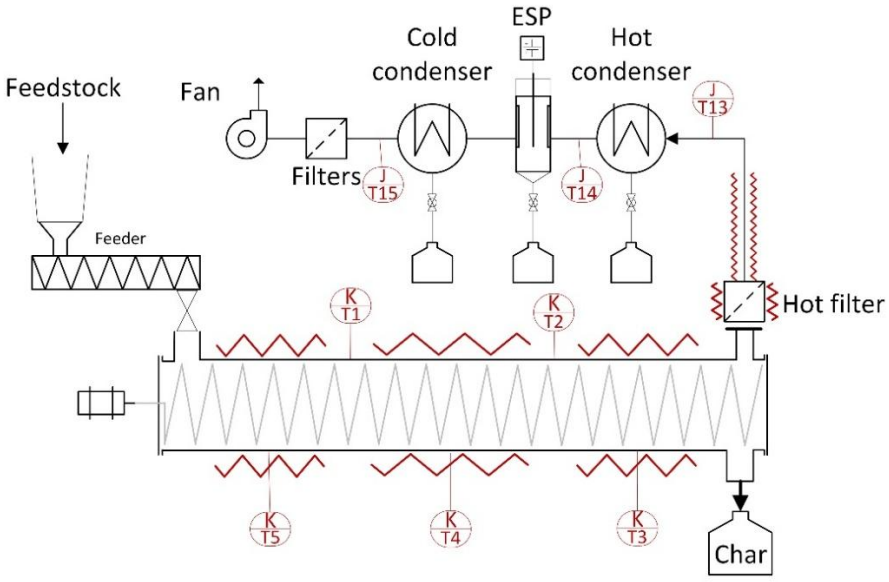


Figure 25: SPYRO basic layout.

Volatiles are separated into a pyrolysis liquid, that is collected in glass bottles, and an off-gas stream composed of permanent gas. Three J-type thermocouples are installed along the pyrogas line to monitor the temperature drop across each condenser. Two pressure transducers are installed on SPYRO, measuring the relative pressures in the reactor and upstream of the blower.

5.2 Feedstock characterization

Poplar was selected for pyrolysis experiments, and its characterization is reported in Table 25. Fixed carbon, volatile matter and ash content of the feedstock were, respectively, 19.2 wt.% (dry base), 80.0 wt.% (dry base) and 0.9 wt.% (dry base). In addition, dry base bulk density and lower heating value were 170 kg m^{-3} and 18.8 MJ kg^{-1}, respectively. Poplars are a valuable source for biofuels and other products, due to their short rotation period (5–8 years), low fertilizer needs, high cellulose and hemi-cellulose content, and low ash content. The feedstock was ground in a Retsch knife mill equipped with an 8 mm sieve and dried for 24 h at 105 °C prior pyrolysis experiments. A picture of the material used for the test is reported in Figure 26.

Table 25: Polar feedstock characterization[34].

Parameter	Value	U.M.
Internal code	RES.22.046.044	
Typology	Wood chips	
Specie	Poplar	
Volatile matter	80.0	%wt db
Ash content	0.9	%wt db
C	50.0	%wt db
H	6.0	%wt db
N	0.2	%wt db
S	0.2	%wt db
O	42.9	%wt db
HHV	20.0	MJ kg^{-1} db
LHV	18.8	MJ kg^{-1} db

Figure 26: Poplar woodchips samples.

5.3 Process description and products yield

The pyrolysis experiment was performed at the operating conditions resumed in Table 26. The maximum temperature was set at 500 °C. The average heating value resulted by the pilot plant operation was about 80 °C min^{-1}.

Table 26: Operating conditions selected for the preliminary pilot production test[34].

Operative conditions	Value	Unit
Reactor temperature	500	°C
Solid residence time	30	min
Biomass flow rate	5	kg/h
Heating rate	80	°C/min

Table 27: Products yields with liquids recovered description[34].

Product yields	Value	Unit
Char	25.6	wt.% db
Light oil phase	2.4	wt.% db
Heavy oil phase	6.7	wt.% db
Aqueous phase	30.3	wt.% db
Demister residues	3.1	wt.% db
Gas (by difference)	31.8	wt.% db

5.3.1 Collection and separation

The condensation unit itself is a multi-stage system[34]. First, a tube-in-tube heat exchanger, operating below 120 °C, condenses and collects the higher molecular weight components.

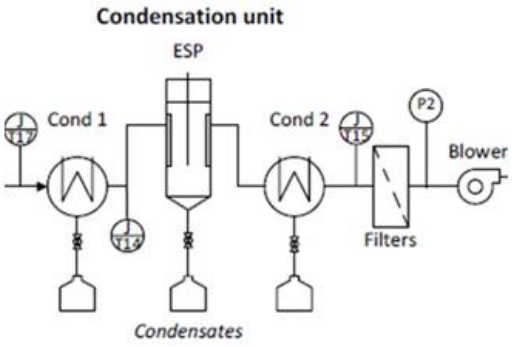


Figure 27: Condensation unit simplified scheme.

Following this, a shell-and-tube heat exchanger, chilled to around 0 °C, facilitates the collection of the majority of the water vapor. Finally, a 12 kV electrostatic precipitator (ESP), powered by a Cascom supply (max 20 kV), removes suspended bio-oil particles (aerosols). As a final aerosol removal step, cotton and metallic wool filters are employed to capture any remaining bio-oil droplets. The collected condensates form the pyrolysis liquid, which is stored in glass bottles, while the remaining permanent gases constitute the off-gas. This liquid mixture naturally separates into an oily phase (OP) and an aqueous phase (APL), with a separating funnel used for more precise density-based separation in the laboratory. A volumetric counter provides a continuous measurement of the permanent gas flow rate during each pyrolysis run. Temperature drops across each condenser are monitored using three J-type thermocouples positioned along the gas line. Furthermore, a T-type thermocouple at the volumetric counter's exit allows for the normalization of the measured gas flow rate.



Figure 28: APL recovery by separation funnel.

5.4 Products characterization

5.4.1 Analytical Methods

The composition of the APL in terms of major organic constituents was analysed through HPLC. The samples were injected in an HPLC apparatus (UFLC Shimadzu) equipped with a refractive index detector, a Hi-Plex column (Agilent) 300 x7.7 mm, operating with 0.005 M sulfuric acid as mobile phase, following the NREL 42623 guidelines. The quantitative analysis was accomplished after a 5-point calibration with pure standards.

The COD (chemical oxygen demand) was determined after oxidation in presence of $K_2Cr_2O_7$ through the photometric COD Cell Test 500-10000 mg/L (COD) (Spectroquant®) and by measuring the absorbance at 605 nm (Shimadzu UV-1800) according to EPA 410.4.
Elemental analysis CHN was performed in a CHN apparatus (LECO Truspec CHN) according to ASTM D5291-21.
The pH was measured in triplicates with a pH meter (Metrohm 827). The pH-meter was calibrated using two buffer solutions of pH 4.0 and 7.0.
The water content determination was performed by Karl Fischer titration (848 Titrino Plus, Metrohm), following ASTM E203-08, titrating about 0.1-0.5 g, with iodine-based Karl Fischer reagents, potentiometrically. The organic content was considered as the complement in weight percentage, considering the inorganic weight contribution negligible.

Figure 29: condensates appearance after dilution to specific acetic acid content (1%wt on the left and 5%wt on the right).

5.4.2 Aqueous phase characterization

The characterization of the aqueous phase of condensate collected is presented in Table 29. Analysis shows an acetic acid content of about 96 mg/l.

Table 28: Aqueous phase characterization[34].

Parameter	**Value**	**U.M.**
Code	RES.23.046.099	
Sample typology	Condensate aqueous phase	
Water content	$wt\%_{wb}$	74.8
TOC	mg/L	65719
pH	-	2.77
C	$wt\%_{wb}$	13.7
H	$wt\%_{wb}$	10.4
N	$wt\%_{wb}$	0.2

O (calc)	$wt\%_{wb}$	75.7
HHV (measured)	MJ/kg wb	6.04
LHV (calc)	MJ/kg wb	3.9
C (calc)	$wt\%_{db}$	54.63
H (calc)	$wt\%_{db}$	7.90
N (calc)	$wt\%_{db}$	0.77
O (calc)	$wt\%_{db}$	36.7
HHV (calc)	MJ/kg_{db}	24.0
LHV (calc)	MJ/kg_{db}	22.4
Acetic acid	mg/l	95.99
Methanol	mg/l	31.20
Glycerol	mg/l	12.12
HMF	mg/l	2.37
Furfural	mg/l	4.99
Catechol	mg/l	5.45
Benzoic acid	mg/l	0.88
Phenol	mg/l	4.46
3-methyl-1,2-cyclop.	mg/l	2.55
3-methoxycatech	mg/l	0.00
Guaiacol	mg/l	0.00

5.4.3 Biochar and oil characterization

The other product obtained were characterized and results are presented in Table 29 about biochar, Table 30 about the light oil fraction and Table 31 about the heavy oil fraction.

- **Biochar**

Table 29: Biochar characterization[34].

Product yields	Value	Unit
Fixed carbon	wt% db	82.3
Volatile matter	wt% db	14.0
Ash	wt% db	3.7
C	wt% db	87.4
H	wt% db	3.1
N	wt% db	0.9
O (by difference)	wt% db	4.8
HHV	MJ/kg db	32.9
LHV	MJ/kg db	32.3
16 PAHs	mg/kg db	5.2
BET area	mq/g	7

- **Light oil fraction**

Table 30: Light oil characterization[34].

Product yields	Value	Unit
Water content	wt%	11.86
Ash	wt%	0.003
C	wt%	58.87
H	wt%	7.51
N	wt%	0.44
O (by diff)	wt%	33.17
HHV (meas)	MJ/kg wb	25.2
LHV (calc)	MJ/kg wb	23.7

- **Heavy oil fraction**

Table 31: Heavy oil characterization[34].

Product yields	Value	Unit
Water content	wt%	11.11
Ash	wt%	0.01
C	wt%	63.04
H	wt%	7.43
N	wt%	0.74
O (by diff)	wt%	28.78
HHV (meas)	MJ/kg wb	28.1
LHV (calc)	MJ/kg wb	26.6

6. Wood vinegar as seed primer

6.1 Seed imbibition

Seed imbibition (or priming) is a physical process driven by the hydraulic conductance of the seed coat and the difference in water potential between the soil and the internal parts of the seed. As a physical process, imbibition is driven by the laws of diffusion and hydraulic flow, with a limited dependence on temperature, while it is more frequently influenced by characteristics such as seed size, density, and sphericity. Seed germination, the first and important phase for plant propagation, starts by seed imbibition, leading to embryo transition from a state of quiescence in a dry seed to a state of highly active metabolism, and terminates with embryonic axis elongation. Generally, seed germination can be divided into three phases: a rapid phase of water uptake (Phase I), followed by a plateau phase of water uptake (Phase II), in which grain morphology and structure obviously change and the radical and germ appear, and post-germination (Phase III), a rapid phase of water uptake with the initiation of growth. During seed imbibition, phase I represents the initial seed germination stage, in which necessary structures and enzymes are present and storage substances, such as starch, proteins, and lipids, which provide energy and nutrition for seed germination, begin to be activated.
Seed priming involves the controlled hydration of seeds with various organic or inorganic chemical solutions[35]. A range of priming techniques, such as hydro-priming, halo-priming, osmo-priming, matrix priming, osmohardening, on-farm priming, hormone or growth enhancer priming, micronutrient seed priming, biopriming (or seed treatment with micro-organisms), and nanoparticle priming (or coating seeds with nanoparticles), are continually being developed to enhance seed quality[36].

6.2 Acetic acid as a seed primer

Acetic acid (**AA**) (CH_3COOH), a simple carboxylic acid, is commonly found as a metabolite in organisms and is the compound responsible for the characteristic sour taste and pungent smell of vinegar, which typically contains 4-5%wt of AA. The use of AA is already well-established for **seed disinfestation**, a common pre-sowing treatment[37,38]. Furthermore, recent studies have highlighted the **potential of acetic acid to enhance plant resistance** to various abiotic stresses, particularly **salinity and drought**. Indeed, while the beneficial effects of AA on osmotic stress tolerance are increasingly recognized, the underlying regulatory mechanism remains to be fully elucidated. It is generally understood that drought stress itself induces acetate accumulation in plants; therefore, the exogenous application of acetate (via acetic acid) is hypothesized to increase drought tolerance across a wide range of plant species (including *Arabidopsis*, rice, maize, wheat, rapeseed, and cassava), suggesting its suitability as an agricultural material[39]. The literature investigating AA as a seed priming agent reveals frequently contradictory results regarding its performance, which are highly dependent on

[35] Kamithi et al., "*Effects of different priming methods and priming durations on enzyme activities in germinating chickpea (Cicerarietinum L.)*" 2016.
[36] Pawar and Laware "*Seed priming: a critical review*" 2018. International Journal of Scientific Research in Review Paper Biological Sciences
[37] Dorna et al., "*The effect of acetic acid treatments on the quality of stored carrot (Daucus carota l.) seeds*" 2021. https://doi.org/10.3390/agronomy11061176
[38] Dorna et al., "*The effect of acetic acid, grapefruit extract and selected essential oils on germination, vigour and health of carrot (Daucus carota l.) seeds*" 2018. DOI: 10.24326/asphc.2018.2.3
[39] Allen and Allen "*Biostimulant potential of acetic acid under drought stress is confounded by ph-dependent root growth inhibition*" 2020. doi: 10.3389/fpls.2020.00647

the concentration used, test conditions, and the specific plant species employed. For instance, the application of acetic acid has been shown to potentiate drought tolerance mechanisms in rice, maize, and wheat through the activation of various physiological and molecular mechanisms[40,41]. A dose of 50 mM of AA was suggested to be effective in mitigating drought adversities in cassava[42] and mung bean (*Vigna radiata*)[43] . Other studies demonstrate positive effects on increasing the germination rate and improving tolerance to environmental stresses (i.e. salinity, drought, cold) using low concentrations: 5-10 mM[44] or 0.5-1 mM[45,46]. Another study investigated the effect of the pH in determining whether AA seed application results in growth promotion or inhibition[47]. They tested on maize seedlings different AA treatments (0, 0.01, 0.1, 1, 10, 20, 29, 38, 47, and 100 mM) spanning a broad range of pH. They observed that growth inhibition was strictly correlated with the concentration of undissociated acetic acid which is pH dependent. These findings led to the key conclusion that low pH exacerbates, and high pH mitigates root growth inhibition in a predictable manner, based on the acetic acid dissociation constant. The undissociated form is lipid soluble and therefore can pass across cell membranes possibly providing negative effect on seed development[47]. In a second experiment, maize seeds were grown in two substrates—a higher-pH potting soil and a lower-pH sphagnum peat—and irrigated with different AA concentrations (0 to 47 mM) without external pH regulation. The buffering capacity of the high-pH soil maintained the pH above 6, keeping AA predominantly in its dissociated (and thus non-membrane-permeable) form. This successfully reduced root damage and significantly mitigated the growth inhibition seen in non-buffered solutions[47].

In brief, following the **main results from the state of the art of acetic acid as seed coating primer** are presented [43,47,48,49]:

- Potential effects: Low concentrations of AA can break seed dormancy, leading to faster and more uniform germination.
- Potential benefits: mild antimicrobial effects on the seed surface and improve early seedling vigor. AA can enhance plant resistance to a variety of abiotic stresses (e.g. salinity and drought).

[40] Kim et al., "*Acetate-mediated novel survival strategy against drought in plants*" 2017. doi: 10.1038/nplants.2017.97
[41] Ogawa et al., "*AA-acid-induced jasmonate signaling in root enhances drought avoidance in rice*" 2021. doi: 10.1038/s41598-021-85355-7
[42] Utsumi et al. "*Acetic acid treatment enhances drought avoidance in cassava (Manihot esculenta)*" 2019. doi: 10.3389/fpls.2019.00521
[43] Rahman et al., "*Acetic acid: a cost-effective agent for mitigation of seawater-induced salt toxicity in mung bean*" 2019. doi: 10.1038/s41598-019-51178-
[44] Zhu, Kunmiao, et al. "*Analysis of the Mechanism of Wood Vinegar and Butyrolactone Promoting Rapeseed Growth and Improving Low-Temperature Stress Resistance Based on Transcriptome and Metabolomics*." *International Journal of Molecular Sciences* 25.17 (2024): 9757
[45] Cai et al., "*Chemical seed priming: molecules and mechanisms for enhancing plant germination, growth, and stress tolerance*" 2020. *Molecules*, *47*(3), 177.
[46] Akter et al., *"Effect of salinity and temperature on the seed germination and seedling growth of desert forage grass Lasiurus scindicus Henr*" 2020. *Sustainability*, *12*(14), 5777.
[47] Amstrong et al., "*Phragmites dieback: sulphide- and acetic acid-induced bud and root death, lignifications, and blockages within aeration and vascular systems*" 1996. doi: 10.1111/j.1469-8137.1996.tb04925.x
[48] Oğuz et al., 2022. The Effect of Acetic Acid Priming on Germination and Sprout Ability of Faba Bean (Vicia faba L.) Seeds. IJIAAR**.** 2022, Vol. 6 (4), 410-416.
[49] Rahman et al., 2023. Acetic acid positively modulates proline metabolism for mitigating PEG-mediated drought stress in Maize and Arabidopsis. FPS. Vol 14, 1167238.

- Variability is key: Effectiveness depends heavily on the plant species and many other different factors (e.g. acetic acid concentration, soaking duration, etc.).
- Mechanisms under study: The exact ways acetic acid promotes germination are still being investigated (hormonal regulation, enzyme activation, seed coat modification).
- AA concentration: wide range tested for pre-sowing seed imbibition, but with promising results were found at very low concentrations: Sub-millimolar up to approximately 10 mM.
    - ➔ **Several studies, using 1 mM concentration, have demonstrated positive effects on increasing the germination rate and improving tolerance to environmental stresses (salinity, drought, cold) using these low concentrations. In our case, to obtain a water solution with 1 mM of acetic acid means a Dilution Factor of about 1600.**
- Soaking time: a general range is from 4 to 12 hours. Optimal soaking time is species-specific. Even slight variations in acetic acid concentration can influence the ideal duration. Environmental conditions during soaking also play a role.
    - It is crucial to emphasize that the optimal concentration and soaking time are highly species-specific and must be determined experimentally

6.3 Wood vinegar as a seed primer

Slow pyrolysis of biomasses, usually in a range of 350-650°C such the processes used in this project, produced char, incondensable gases and condensable vapours. The latter were condensed into pyrolysis liquids which could be separated in an organic fraction (pyrolysis oil) and an APL (Aqueous pyrolysis liquid) composed mainly by water and water-miscible organic compounds. **The APL also known as wood vinegar when produced from lignocellulosic biomasses is a complex mixture containing hundreds of different organic compounds**. The exact composition can vary depending on the type of biomass used, the temperature of pyrolysis, the oxidation grade and the condensation methods. The main APL constituents besides water are organic acids (acetic acid is usually one of the main constituents), phenols, cresols, alcohols (such as methanol) esters and furfurals.

This byproduct of biomass pyrolysis finds diverse potential applications, primarily in agriculture, where it has been reported to act as a natural soil amendment[50], enhancing microbial activity and nutrient availability, while also serving as a bio-pesticide[51] to repel certain insects and inhibit fungal and bacterial diseases in plants[52]; furthermore, it can stimulate plant growth[53], improve seed germination, and even suppress weeds under specific conditions[54], and its odour-absorbing qualities make it useful for managing smells in animal husbandry and composting[55].

[50] Akley, Edwin K., et al. "*Wood vinegar promotes soil health and the productivity of cowpea*." *Agronomy* 13.10 (2023): 2497.
[51] Akkuş, Memiş, Çağlar Akçay, and Mesut Yalçın. "*Antifungal and larvicidal effects of wood vinegar on wood-destroying fungi and insects*." *Maderas. Ciencia y tecnología* 24 (2022).
[52] Iacomino, Giuseppina, et al. "*Exploring the potential of wood vinegar: Chemical composition and biological effects on crops and pests*." *Agronomy* 14.1 (2024): 114.
[53] Zhu, Kunmiao, et al. "*Analysis of the Mechanism of Wood Vinegar and Butyrolactone Promoting Rapeseed Growth and Improving Low-Temperature Stress Resistance Based on Transcriptome and Metabolomics*." *International Journal of Molecular Sciences* 25.17 (2024): 9757.
[54] https://www.interregeurope.eu/good-practices/wood-vinegar-as-natural-bio-herbicide#further-information
[55] Takahara, Y., et al. "*Study on odor control using wood vinegars*." *[Nihon Koshu Eisei Zasshi] Japanese Journal of Public Health* 40.1 (1993): 29-38.

As a soil amendment, wood vinegar can positively influence the soil microbiome by providing readily available carbon sources and potentially stimulating the growth and activity of beneficial microorganisms [56,57]. This enhanced microbial activity can lead to improved nutrient cycling, making essential elements more accessible to plants [58]. Furthermore, the acidic nature of wood vinegar, along with its diverse organic constituents, can aid in the solubilization of certain soil nutrients, further boosting their availability for plant uptake[59].

Beyond its soil-enhancing properties, wood vinegar has demonstrated potential as a bio-pesticide [5]. Its volatile organic compounds can act as repellents against certain insect pests, disrupting their behaviour or deterring them from feeding on crops. Additionally, the presence of phenolic compounds and acetic acid contributes to its ability to inhibit the growth of various fungal and bacterial pathogens that can cause plant diseases[60]. This dual action of promoting plant health and offering a degree of protection against pests and diseases makes wood vinegar a valuable tool in integrated pest management strategies.

The application of wood vinegar can also directly influence plant physiology and development**. Studies have shown that diluted solutions of wood vinegar can stimulate seed germination, leading to faster and more uniform seedling emergence**[61]. Moreover, it can promote vegetative growth, potentially increasing plant vigour and overall biomass production. Interestingly, under specific conditions and application rates, wood vinegar has also exhibited the ability to suppress weed growth, possibly through allelopathic effects or by altering the soil environment in a way that is unfavourable to weed germination and establishment[62].

The unique chemical composition of wood vinegar also lends itself to odour management in agricultural settings. Its ability to react with and neutralize ammonia and other volatile compounds responsible for unpleasant odours makes it a useful tool in animal husbandry, where it can help improve air quality in livestock facilities[63]. Similarly, its application in composting can aid in reducing foul smells associated with the decomposition of organic matter, leading to a more manageable and less offensive process also for composting processes[64].

Beyond its uses in agriculture, ongoing research is exploring and uncovering further potential applications for wood vinegar and its individual constituents. Investigations are underway in areas such as food preservation, where its antimicrobial properties could be harnessed to extend the shelf life of food products [65]. Furthermore, its complex mixture of organic compounds is being examined for

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[56] Gu, Shiguo, et al. "N*ew insights into the impact of wood vinegar on the growth and rhizosphere microorganisms of cherry radish (Raphanus sativus L.)*." *PeerJ* 12 (2024): e18505.

[57] Akley, Edwin K., et al. "*Wood vinegar promotes soil health and the productivity of cowpea*." *Agronomy* 13.10 (2023): 2497.

[58] Yang, Liu, et al. "*Effect of combined application of wood vinegar solution and biochar on saline soil properties and cotton stress tolerance*." *Plants* 13.17 (2024): 2427.

[59] Theapparat, Yongyuth, et al. "*Physicochemical characteristics of wood vinegars from carbonization of Leucaena leucocephala, Azadirachta indica, Eucalyptus camaldulensis, Hevea brasiliensis and Dendrocalamus asper*." *Agriculture and Natural Resources* 48.6 (2014): 916-928.

[60] Nutsukpo, Efoo Bawa, et al. "*Grapevine Response to Pyroligneous Acid: Antifungal, Physiological, and Biochemical Impacts*." *Crops* 5.2 (2025): 21.

[61] Abdolahipour, B., and M. Haghighi. "*Effect of pine wood vinegar on germination, growth and physiological characteristics, and uptake of elements in basi*l." (2019): fa11-fa23.

[62] Chu, Lei, et al. "*Evaluation of wood vinegar as an herbicide for weed control*." *Agronomy* 12.12 (2022): 3120.

[63] Hagner, Marleena, et al. "Slow pyrolysis liquid in reducing NH3 emissions from cattle slurry—Impacts on plant growth and soil organisms." Science of the Total Environment 784 (2021): 147139.

[64] Zhu, Rixing, et al. "Biochar and pyroligneous acid contributed to the sustainable reduction of ammonia emissions: From compost process to soil application." *Journal of Hazardous Materials* 489 (2025): 137677.

[65] Wang, Jiaqing, Irina Potoroko, and Lina Tsirulnichenko. "Wood vinegar and chitosan compound preservative affects on fish balls stability." *Food Bioscience* 42 (2021): 101102.

potential use as a cosmetic ingredient[66] or as a component in animal feed supplements[67], suggesting a broader range of valuable applications beyond the agricultural realm.

6.4 Selected species for germination trials

For the germination trials (see next paragraph), three model species were chosen: castor, sorghum and safflower. **These species were selected for their relevance to the MIDAS project and because they represent an important category of biomass species suitable to grow in marginal soils with different constraints**.
While castor, sorghum and safflower belong to different botanical families, have varying growth cycle and are used for different purposes, they can have difficulties with proper germination due to diverse physiological, morphological and environmental reasons. Inappropriate germination could significantly reduce yield and biomass productivity during field cultivation, particularly when grown in marginal land or under environmental stress, leading to increase the cultivation cost at farm scale. Selecting the most suitable genotype is a crucial step for choosing the variety that performs best in a specific environment for production. However, it is also vital to investigate every possible strategy to improve seed germination and enhance crop productivity. This research aims to investigate the effect of recovered-based acetic acid solution used as seed primer strategy on seed germination and quality.

- **Castor bean (Ricinus communis L.)**

Castor is an important industrial crop producing oilseeds for biofuel production. Castor bean germination is slow and uneven, leading often to poor crop establishment [68]. This uneven emergence results in a high variability in plant age, preventing late-emerging seedlings from reaching their full yield potential and complicating farm operations like weed management and mechanical harvest. Furthermore, the persistence of viable seeds in the soil emerges over an extended period in subsequent seasons[69]. Castor scarce germinability is primarily related to the environmental stresses typical of semi-arid regions, particularly drought and soil salinity[70]. These factors reduce the soil water potential and limit seed water uptake. Furthermore, excessive salinity led to phytotoxic effects due to the absorption of ions that further compromise crop development[71]. In addition, form the physiological point of view, the seed coat plays an important role in influencing the germinability performance**.** Germination seems not to be delayed because of restrictions to hydration, but related to the mechanical resistance to the embryo expansion[68]. This physical barrier restricts the expansion of the embryo, a constraint that can keep the seed arrested in the full hydration stage for many days without progressing to radicle protrusion. This mechanical effect can be modulated by the variety morphological features, with the seed coat relative weight (the ratio of seed coat weight to total

[66] Kim, Tagon, et al. "Anti-oxidation and anti-atopic dermatitis effect of herbal wood vinegar." *Korean Chemical Engineering Research* 48.6 (2010): 690-694.
[67] Sureshkumar, Shanmugam, Vetriselvi Sampath, and In Ho Kim. "The influence of dietary inclusion of wood vinegar supplementation on growth performance, nutrient digestibility, and meat quality in grower-finisher pigs." *Acta Biochimica Polonica* 68.2 (2021): 287-292.
[68] Severino "*Studies on the Germination and Emergence of Castor Seedlings*" 2024. ttps://doi.org/10.3390/seeds3020019
[69] Sun et al., "*Seedling emergence, growth, and leaf mineral nutrition of Ricinus communis L. cultivars irrigated with saline solution*" 2013.
[70] Cunha et al., "*Castor (Ricinus communis L.) differential cell cycle and metabolism reactivation, germinability, and seedling performance under NaCl and PEG osmoticum: Stress tolerance related to genotype-preestablished superoxide dismutase activity*" 2024. doi: 10.1016/j.plaphy.2024.108372.
[71] Gong et al., "*Effects of the interaction between biochar and nutrients on soil organic carbon sequestration in soda saline-alkali grassland: A review*" 2021. https://doi.org/10.1016/j.gecco.2020.e01449

seed weight) identified as the most critical factor influencing the time required for germination. Specifically, faster germination is observed in seeds that possess a smaller volume, higher volumetric density, and, crucially, a thinner seed coat[68].

Figure 30: Castor been seeds supplied by CRES.

- **Sorghum (Sorghum bicolor L.)**

Sorghum is the best alternative to corn for bioenergetic purposes and animal feeding due to its greater adaptability, higher drought resistance, and lower nutritional demands cereal crop. Sorghum exhibits intraspecific variability for the rate of dormancy release and pre-harvest sprouting behaviour[72]. This primary issue is rooted in seed dormancy, an inherent protective mechanism that prevents seeds from germinating even when environmental conditions are favourable. By contrast, genotypes with low level of dormancy prior to the harvest have been affected by susceptibility to pre-harvest sprouting. Sorghum dormancy is a complex, genetically regulated process imposed by the intricate interaction between the outer covering tissues and the embryo itself[73]. The covering structures play a predominant role in the overall dormancy level, partly due to the accumulation of various phenolic compounds[73]. At the physiological level, the switch between dormancy and germination is governed by two key plant hormones: abscisic acid, which promotes dormancy, and gibberellins, which trigger germination. Generally, seeds maintain dormancy through high abscisic acid and low active gibberellins levels. However, the specific mechanism for dormancy maintenance in sorghum deviates somewhat from the general cereal model. Research indicates in sorghum the crucial factor is the embryo's sensitivity to abscisic acid rather than the absolute content of endogenous abscisic acid within the seed [72,73].

[72] Rodrıguez et al., "*Expression of seed dormancy in grain sorghum lines with contrasting pre-harvest sprouting behavior involves differential regulation of gibberellin metabolism genes*"2011. doi:10.1093/pcp/pcr154

[73] Benech-Arnold and Rodríguez "*Pre-harvest sprouting and grain dormancy in sorghum bicolor: what have we learned*?"2018. https://doi.org/10.3389/fpls.2018.00811

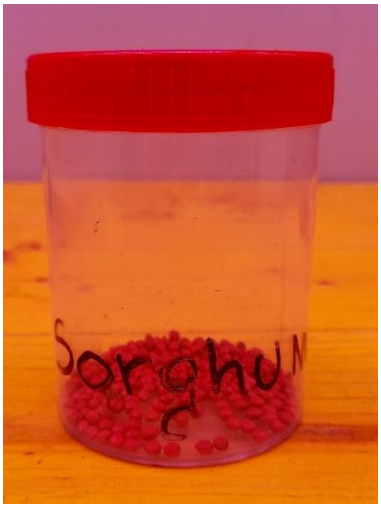


Figure 31: Sorghum seeds supplied by CRES.

- **Safflower (Carthamus tinctorius L.)**

Safflower is an important industrial crop with a wide range of destinations including food, cosmetics, and biorefinery. Generally, safflower exhibits high seed viability and is not considered to suffer from significant issues related to dormancy. The perception of poor germination in safflower is usually attributed to suboptimal environmental conditions rather than a biological limitation of the seed itself**.** Optimal soil moisture, temperature shallow planting depth, and high-quality seed are the critical factors determining a rapid and uniform emergence. Safflower, however, is a species well-suited for cultivation on difficult, marginal lands under suboptimal water availability. In this context, preliminary seed imbibition could be an effective, low-cost strategy to bypass initial germination issues and promote better field emergence. Improving the establishment phase in such harsh environments would subsequently lead to a more uniform crop stand and ultimately enhance the overall yield and production potential of safflower.

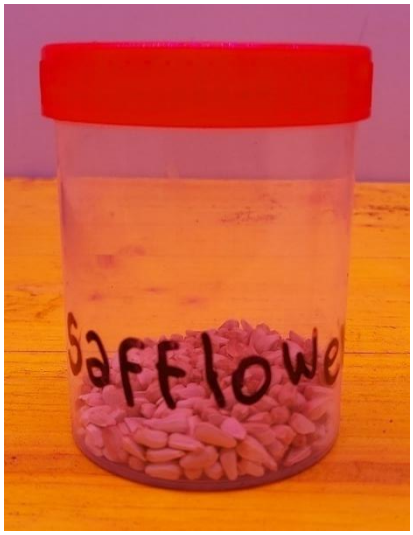


Figure 32: Safflower seeds supplied by CRES.

The use of acetic acid for stimulating germination remains a topic requiring significant further investigation and substantial protocol optimization. This optimization must be precisely tailored to specific growing conditions and the plant species employed, focusing critically on determining the optimal effective dose. The following sections will introduce the **acetic acid-based solutions**

which are derived from the **aqueous phase of the slow pyrolysis of wood chips** and the **species tested within the scope of the MIDAS Project**.

6.5 Materials and methods for germination tests

The germination experiments were conducted using **germination trays** featuring 12 individual cells with a common base tray, equipped with a clear lid and an adjustable air vent (see Figure 33). The **substrate** used consisted of a mixture of neutral **sphagnum peat**, **green compost**, and **sand** (Table 32). To ensure uniformity and prevent clumping, the substrate was mildly ground to avoid conglomerates and subsequently sieved using a 2 mm mesh (Figure 34). Each cell was filled with 25 grams of substrate, resulting in a substrate water capacity (Field Capacity, FC) of 24 ml per cell.

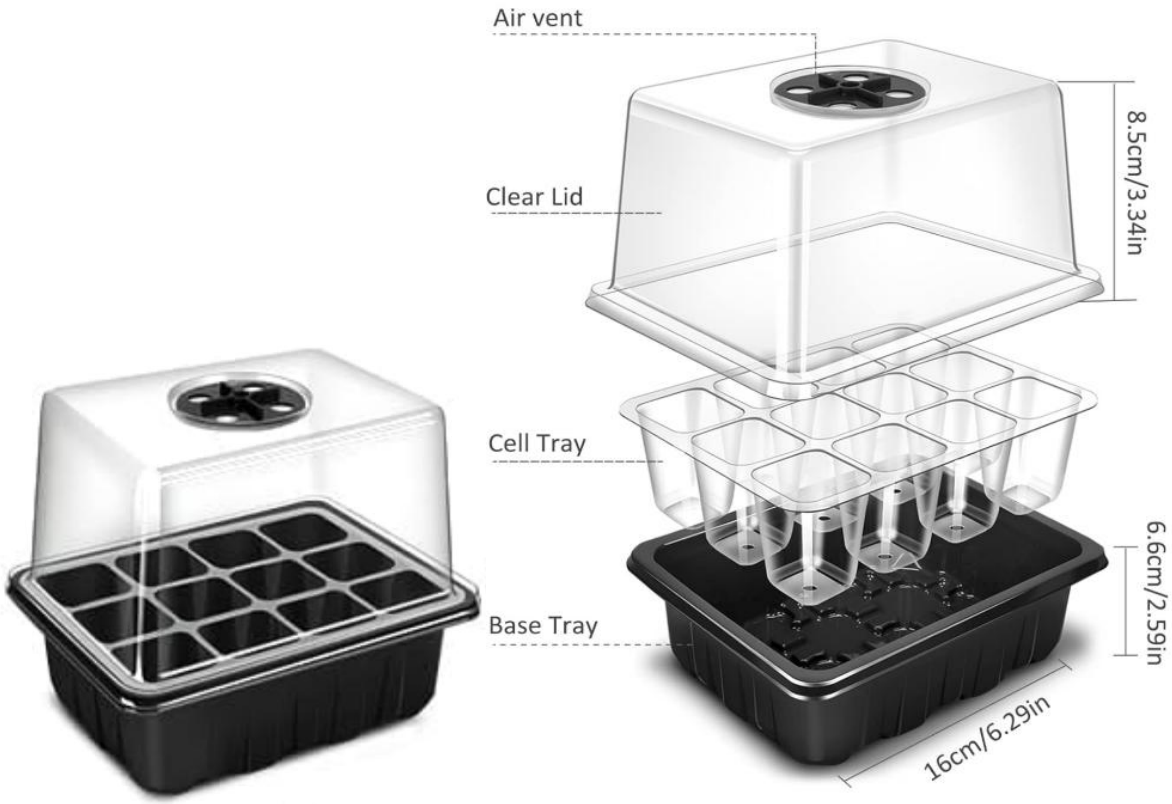


Figure 33: Germination tray with 12 cells, dimensions.

Table 32: Substrate characteristics.

Basic cultivation substrate	Value	Unit
pH (in H_2O)	7	-
Electrical conductivity	0.6	dS/m
Total porosity	80	%

Figure 34. Commercial substrate as received (left), mild grinded(middle), sieved and finally put in the cell (25 g per cell) and irrigated (right).

Depending on the species and test, four distinct **substrate water capacity regimes** were established and maintained throughout the experiment using distilled water, calculated based on the Field Capacity (FC) of the substrate:

- 24 ml per cell means 100% of the field capacity (100FC)
- 18 ml per cell means 75% of the field capacity (75FC)
- 12 ml per cell means 50% of the field capacity (50FC)
- 6 ml per cell means 25% of the field capacity (25FC)

Sowing was performed by placing one imbibed seed per cell slightly under the surface of the substrate. Following sowing, the desired Field Water Capacity (e.g., 75%, 50%, or 25% FC) was achieved by adding the required amount of distilled water using a graduated syringe (0.5 ml graduation). An **evaporation control treatment** was used to accurately measure water loss via weight control every two days per week. These control trays were kept covered with the clear lid and the air vent open, simulating the conditions of the test trays. The average weight loss of triplicate control trays (relative to Day 0) was calculated for each water regime (e.g., 75%, 50%, and 25% FC). This value was then divided by 12 cells to determine the exact amount of water lost per cell. This calculated water loss was then added back to all treatment cells using distilled water delivered via a graduated syringe to restore the target water content. The final volume of added water was rounded up or down to the nearest value in terms of half a milliliter for practical application.
For the imbibition, a condensate solution was prepared and diluted to achieve a final acetic acid concentration of 1 mM/L. The seed imbibition process lasted for 5.5 hours. This was performed in a plastic jar containing 15 grams of seeds and 80 ml of either distilled water or the diluted condensate. Manual mixing was performed every 2 hours. Following imbibition, seeds underwent a natural drying process in the dark for approximately 12 hours.
The experimental trays were monitored twice per week. During these visits, germination was recorded, and the water content was verified. Photographic records were taken during every monitoring visit.

6.5.1 Seed imbibition protocol

1. Solution preparation (dilution stage)
Goal: Prepare a final solution with an Acetic Acid content of 1 mM/L.
Initial Inventory: 50 ml of received wood vinegar (2.5%wt Acetic Acid content).

Required Dilution Factor (DF): 416.
Final volume: The received 50 ml will yield approximately 20.8 liters of final solution.
Procedure:

a. Measure the required volume of wood vinegar (WV) from the 50 ml stock.
b. Add 1 ml of the original WV stock to 415 ml of distilled water. (Ratio 1:416).
c. Mix thoroughly to obtain the final working solution (1 mM/L Acetic Acid).

2. Seed imbibition
Procedure:

a. Place all seeds intended for treatment into a container (box).
b. Pour the prepared working solution over the seeds, ensuring they are completely submerged (covered).
c. Allow the seeds to soak (imbibe) in the solution for a total duration of 5 hours and 30 minutes.

3. Drying and storage
Procedure:

a. After the 5.5-hour imbibition period, carefully remove the seeds from the solution.
b. Spread the treated seeds onto a drying surface (e.g., cloth or paper) in the open air.
c. Allow the seeds to dry overnight (12 hours).

4. Sowing

6.6 Preliminary tests

Germination tests for T4.2.4 were realized at the RE-CORD climatic chamber within the experimental area at Montepaldi (San Casciano Val di Pesa, Florence, Italy).

A preliminary test was conducted to verify the effect of acetic acid (AA) and poplar wood vinegar (WV) agent-based solutions on maize germination. In this test, the same solutions used for seed priming were also used for irrigation.
Three types of imbibition-based treatments were assessed using distilled water, acetic acid and poplar wood vinegar both with an acetic acid concentration of 1 mM/l. Two water stress were tested: 75% and 50% of the field capacity. Moreover, two types of irrigation were tested, one with distilled water and the second with acetic acid or poplar wood vinegar both with an acetic acid concentration of 1mM/L.
The tests were carried out in germination chambers using a commercial peat-based potting mix with a pH of 7, previously sieved to 2 mm (Figure 35).

Figure 35: Germination cells, and peat substrates used for the trials .

Table 33. Summary of the treatments. illustrates a summary of the treatments investigated in this trial.

*Table 33. Summary of the treatments. * AA= acetic acid; WV = wood vinegar; C= control.*

Treatments	Seed imbibition	Irrigation type	Water regime
C	distilled water	distilled water	75% FC
C-AA	acetic acid 1mM	distilled water	
C-WV	wood vinegar 1mM	distilled water	
AA	acetic acid 1mM	acetic acid @1mM	
WP	wood vinegar 1mM	wood vinegar @1mM	
C	distilled water	distilled water	50% FC
C-AA	acetic acid 1mM	distilled water	
C-WV	wood vinegar 1mM	distilled water	
AA	acetic acid 1mM	acetic acid @1mM	
WV	wood vinegar 1mM	wood vinegar @1mM	

Two additional trays were established, namely P-75% and P-50% (Figure 36). These two trays were used to assess the amount of water lost through evaporation. Irrigation was based on assessing the water content of the two P trays. Whenever the moisture content of the P tray fell below the level equivalent to the field capacity at 75% and 50%, it was irrigated in order to keep the moisture content of the treated trays constant.

The imbibition lasted 5.5 h, after which the seeds were allowed to dry for one night. Sowing was done the next day in the germination trays (each treatment involved the sowing of 12 seeds in a tray). Before sowing, each slot was saturated with distilled water to facilitate germination.

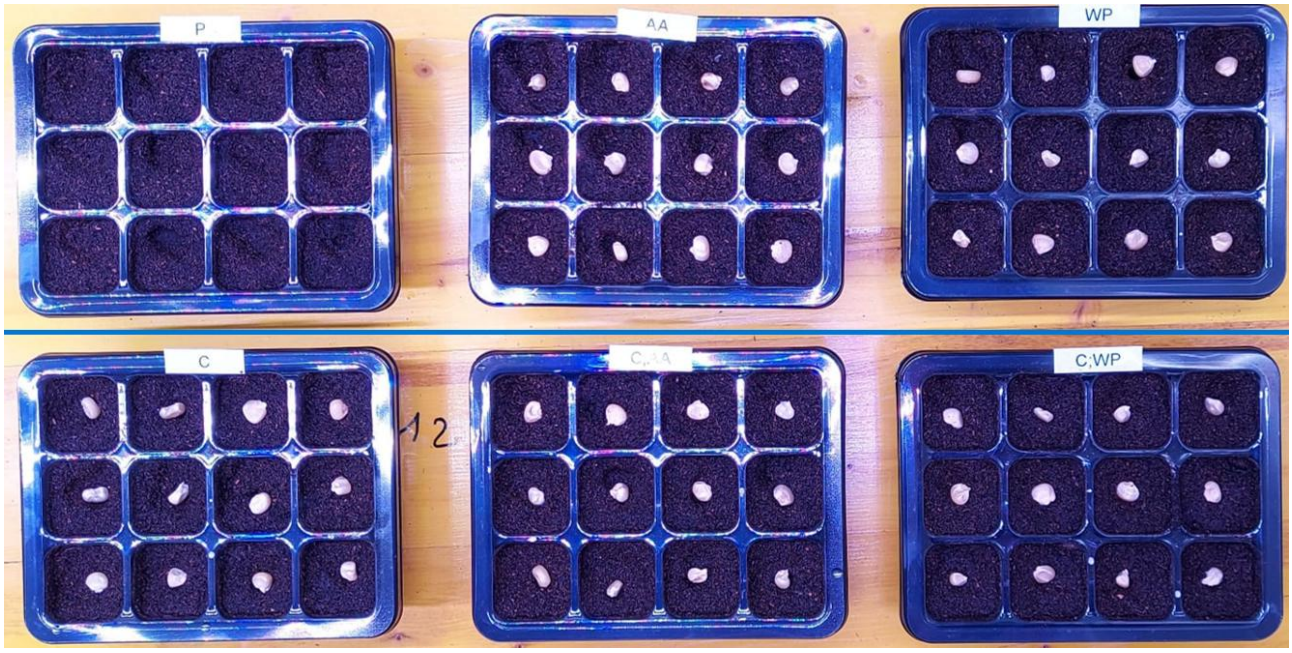


Figure 36. Treatment trays during sowing. P tray was used for assessing the amount of water lost through evaporation.

6.6.1 Results and preliminary considerations

Table 34 shows the results of the germination test. In general, treatments receiving the 75% of the field capacity showed a better performance, and in particular the highest germinability was observed for C-WV and AA, reaching 67% of germinated seed. Compared to these two latter, the control C treatment showed a lower germination rate of 25%, and this result may indicate that imbibition with wood vinegar and acetic acid improved maize germinability. Under water stress, treatments behave differently. The highest germination rate was observed for seeds imbibed and irrigated with AA and WV (Table 34). Finally, the C-WV treatment, primed with wood vinegar and irrigated with distilled water, showed a slightly faster germination rate compared to the control (C) under both water regimes. This result confirms the suitability of this solution for use as a priming agent. In subsequent trials, the diluted condensation agents will only be tested as primers to prevent any overlapping effect with irrigation.

*Table 34. Percentage of germinated seeds during the trial with maize. *DAS= days after sowing. * AA= acetic acid; WV = wood vinegar; C= control.*

Treatments	% of germinated seeds		
	Seed imbibition	Irrigation type	Water regime
C	distilled water	distilled water	75% FC
C-AA	acetic acid 1mM	distilled water	
C-WV	wood vinegar 1mM	distilled water	
AA	acetic acid 1mM	acetic acid @1mM	
WP	wood vinegar 1mM	wood vinegar @1mM	
C	distilled water	distilled water	50% FC
C-AA	acetic acid 1mM	distilled water	
C-WV	wood vinegar 1mM	distilled water	
AA	acetic acid 1mM	acetic acid @1mM	
WV	wood vinegar 1mM	wood vinegar @1mM	

		% of germinated seeds				
Water regime	Treatments	10DAS	12DAS	14DAS	16DAS	19DAS
75% FC	**C**	25	25	25	25	25
	C-AA	17	25	25	25	25
	C-WV	33	58	67	67	67
	AA	17	58	58	67	67
	WV	8	17	17	17	17
50% FC	**C**	0	17	25	25	33
	C-AA	0	0	0	0	0
	C-WV	0	8	17	25	25
	AA	8	42	50	50	50
	WV	17	42	50	50	50

6.7 Germination tests

The object of this trial was to evaluate the germination performance of castor, sorghum and safflower seeds, soaked with condensate and subjected to different irrigation conditions. This will help assess seeds' resilience and performance under both optimal and water-stressed conditions.

The solution used for the imbibition was realized diluting poplar wood vinegar with distilled water to obtain a solution with 1 mM/L acetic acid content. In total, three treatments were tested:

- Control treatment (**C**): no imbibition
- Water treatment (**W**): imbibition in distilled water
- Wood vinegar treatment (**WV**): imbibition in wood vinegar (1 mM/l of AA)

The imbibition process was done for safflower, castor and sorghum seeds, as shown in Figure 37. The process lasted for 5.5 hours, using 80 ml of water or condensate diluted, added to a plastic jar with 15 gr of seeds (Figure 37). Manual mixing every 2 hours, before a natural seed drying in the dark of about 12 hours.

Figure 37. Safflower, castor bean and sorghum during imbibition in distilled water and diluted poplar wood vinegar.

Furthermore, three different water regime treatments were tested:

- Water irrigation maintaining 75% of the field capacity (FC)
- Water irrigation maintaining 50% of the field capacity (FC)
- Water irrigation maintaining 25% of the field capacity (FC)

The three water regimes were applied in sorghum and safflower trials, while for castor bean only the 75% and 50% water treatments were tested.
Additional trays (namely EVA75, EVA50 and EVA25) without seeds, were set up to monitor daily evaporation for maintaining the desired moisture content.
The tests were carried out in germination chambers using a commercial peat-based potting mix with a pH of 7, previously sieved to 2 mm (Figure 35).
Each treatment included the sowing of 12 seeds per one tray. In total 3 trays were prepared for each treatment; 36 seeds were sown for each crop. Climatic chamber parameters were set with a 12/12-hour light/dark cycle and an average temperature of 24°C. Monitoring occurred twice per week, encompassing the assessment of germination and water content, and included taking pictures during every monitoring visit.

6.7.1 Results

- **Castor bean**

After the pre-sowing imbibition (see Figure 45) with water only (W) and diluted wood vinegar (WV), the trial germination started.

Figure 38. Castor seeds pre-sowing imbibition with water only (right) and wood vinegar (left).

High variability in seed germination was observed in the castor treatment from the initial stages of the experiment (Figure 39). For this reason, it was not possible to perform statistical analysis using ANOVA. However, to understand the effect of the treatments, the number of germinated seeds was processed and converted into a percentage and graphs are shown below.

Figure 39. Castor bean seed in germination during the trial.

The duration of the trial lasted about 37 days, with the first monitoring occurring 9 days after sowing. Following results are presented graphically (showing the percentage of castor seed germinated) and then commented:

- **Water stress influence on the germination rate**

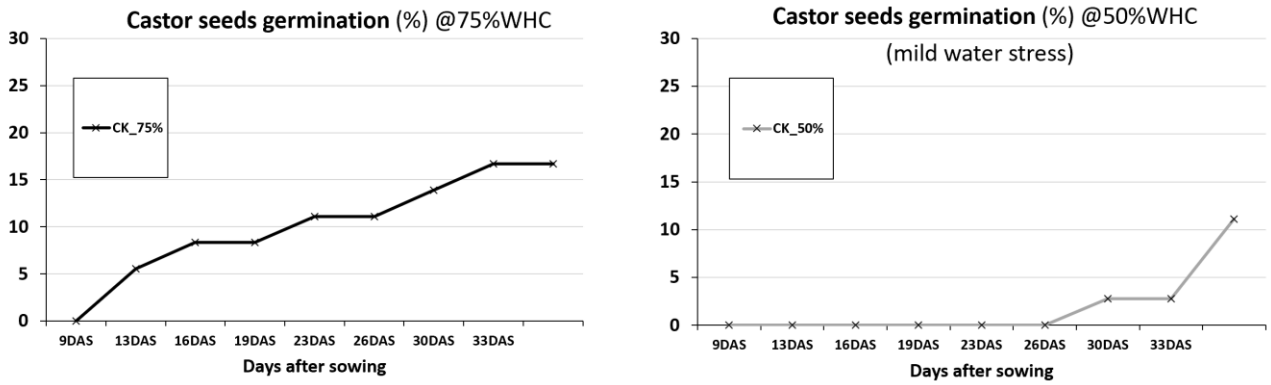


Figure 40. Water stress influence on the germination rate of castor beans.

Control resulted in very low germination rates in both the treatments (due to the castor variety supplied). Water stress condition affected the seeds response with a **delay in germination and with a less germination rate**

- **Water stress imbibition influence on the germination rate**

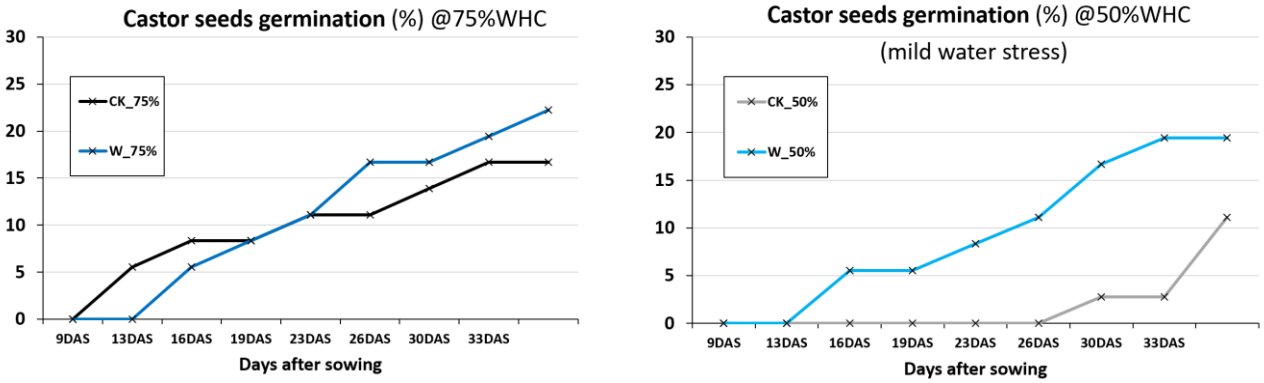


Figure 41. Water imbibition influence on the germination rate of castor beans.

Water imbibition improved the germination rate in both the water regime, reaching under water stress similar results obtained in the 75%SC.

- **Wood vinegar imbibition influence on the germination rate**

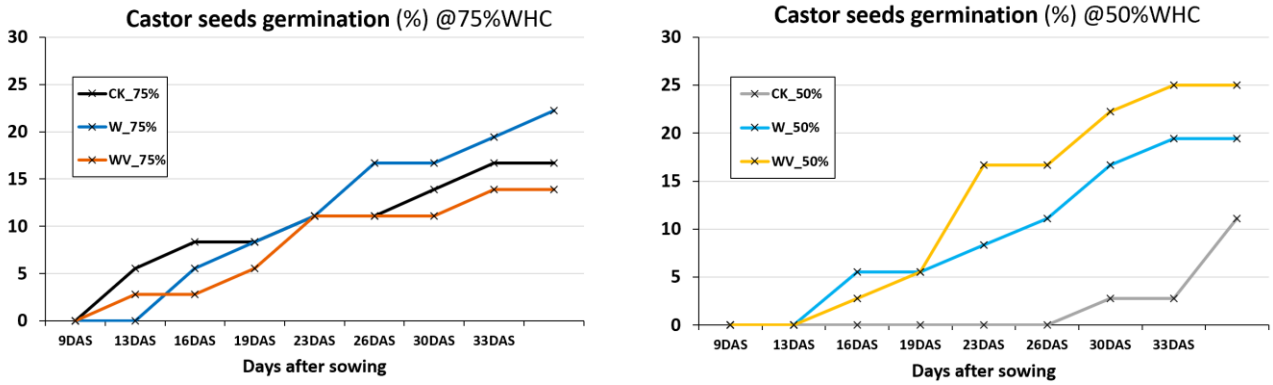


Figure 42. Wood winegar imbibition influence on the germination rate of castor beans.

Water imbibition improved the germination rate in both the water regime, reaching under water stress similar results obtained in the 75%SC. Wood vinegar imbibition resulted with the highest germination rate, under water stress condition, anticipating also the timing of germination.

- **General imbibition effect on germination reate**

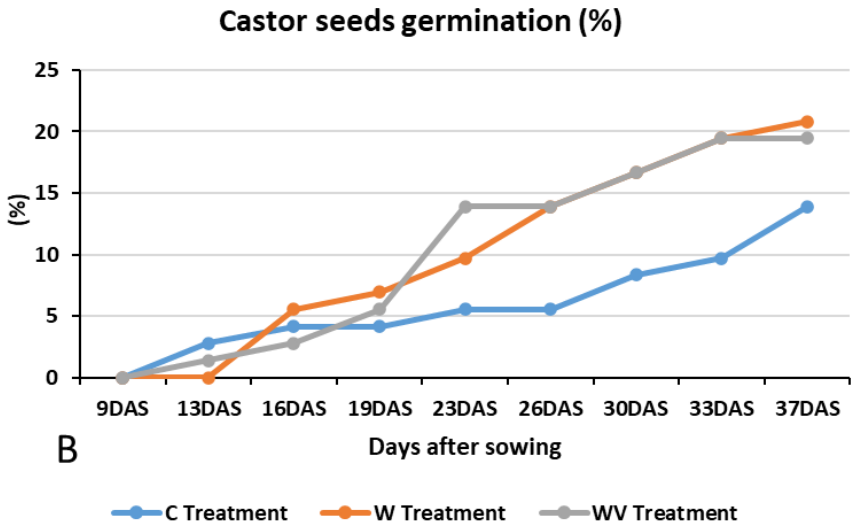


Figure 43. Percentage of castor seeds germinated throughout the germination test. Figure A shows the effect of the water treatments (i.e. 75% and 50% of the field capacity), while figure B shows the effect of the imbibition treatments (B = no imbibition, W= water imbibition, C = wood vinegar imbibition).

Regarding the effect of the type of imbibition (Figure 43B), after 19 days from sowing it was possible to observe a clear difference among the imbibed treatments (W and WV treatments) and control treatment. The imbibed treatments showed a higher germination which lasted till the end of the experiment. These results indicates that seeds imbibition ether with distilled water or wood vinegar, improved the germinability of castor seeds.

Figure 44 shows the average (and standard deviations) of the germinated seeds for the treatments at the end of the monitoring period. The treatment involving imbibition with wood vinegar under water stress was the one that performed best, also showing the lowest variability. This result is particularly interesting because it suggests that the imbibition process was effective in promoting germination and that the compounds in the wood vinegar may have simultaneously enhanced seed vigor and conferred initial tolerance to subsequent environmental conditions.

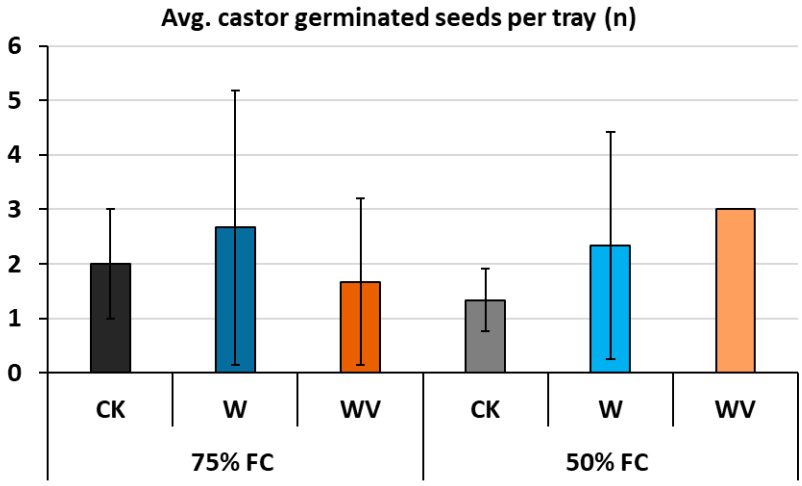


Figure 44: Mean of the castor seeds germinated (n=3) during the last day of monitoring. In each histogram, the vertical bar represents the standard deviation.

- **Sorghum**

After the pre-sowing imbibition (see Figure 45) with water only (W) and diluted wood vinegar (WV), the trial germination started.

Figure 45: Sorghum seeds (left) and pre-sowing seeds imbibition with water only (centre) and wood vinegar (right).

The germination test with sorghum seeds last 9 days. Sorghum exhibited a high germination rate, with the percentage of germinated seeds ranging between 67% and 92%, achieved within 9 days. Although no significant differences were observed, the highest germination percentages were achieved with seeds irrigated at 75% and 50% of the field capacity (Figure 47).

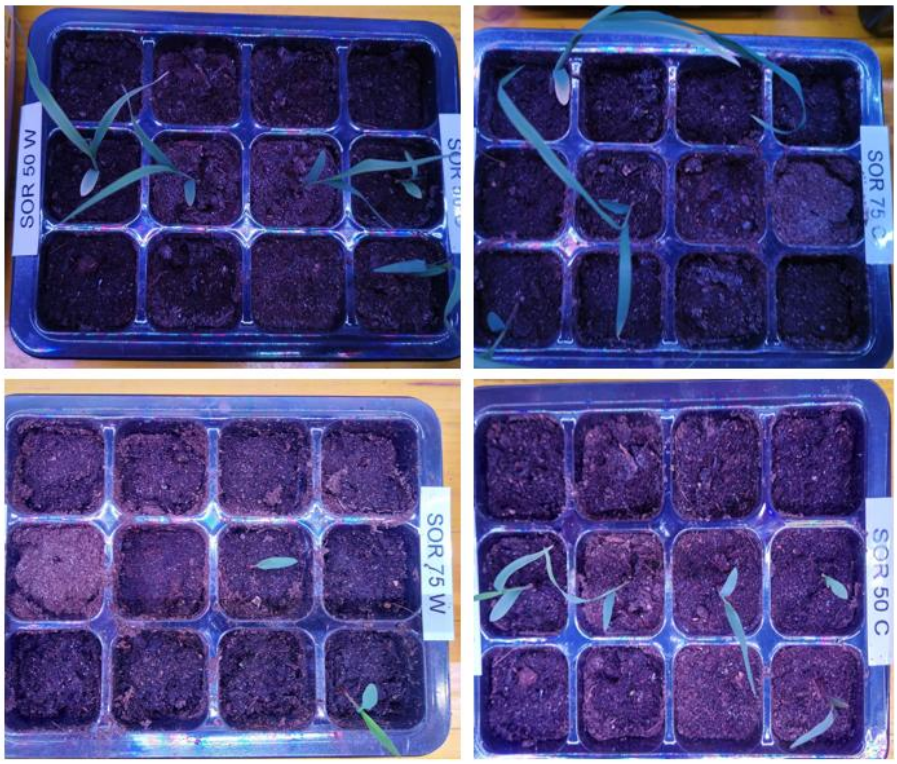
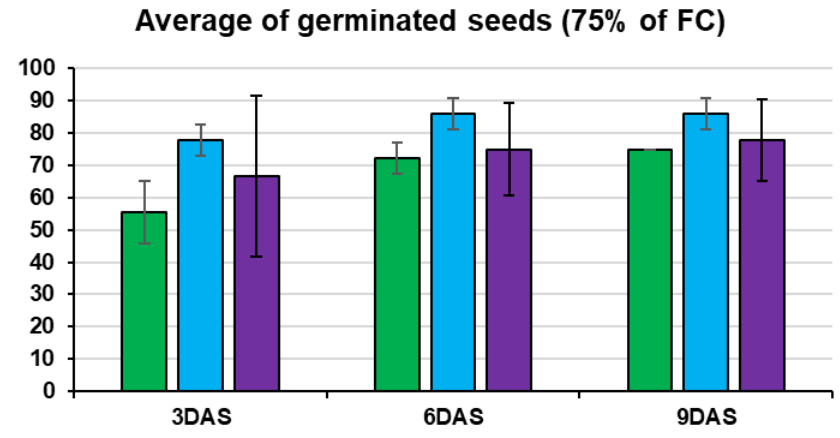
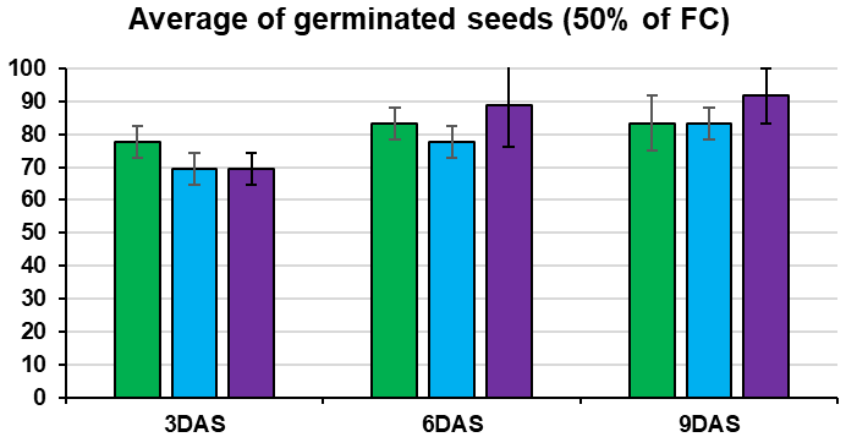


Figure 46: Sorghum seed in germination during the trial.

Regarding the type of imbibition, no significant differences were observed among the three treatments and no inhibitory effects on germination were observed in seeds treated with the wood vinegar solution.





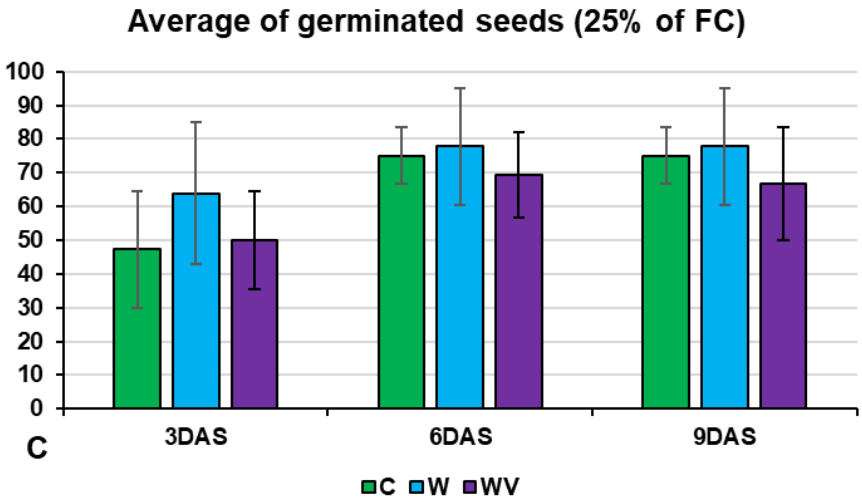


Figure 47. Average of sorghum germinated seeds measured at 3, 6 and 9 days after the sowing. In each histogram, the vertical bar represents the standard deviation.

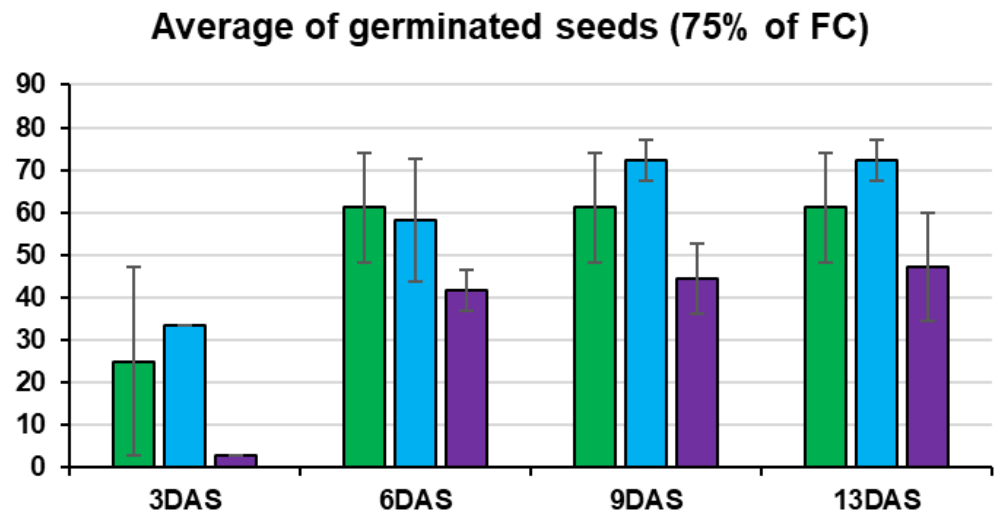
- **Safflower**

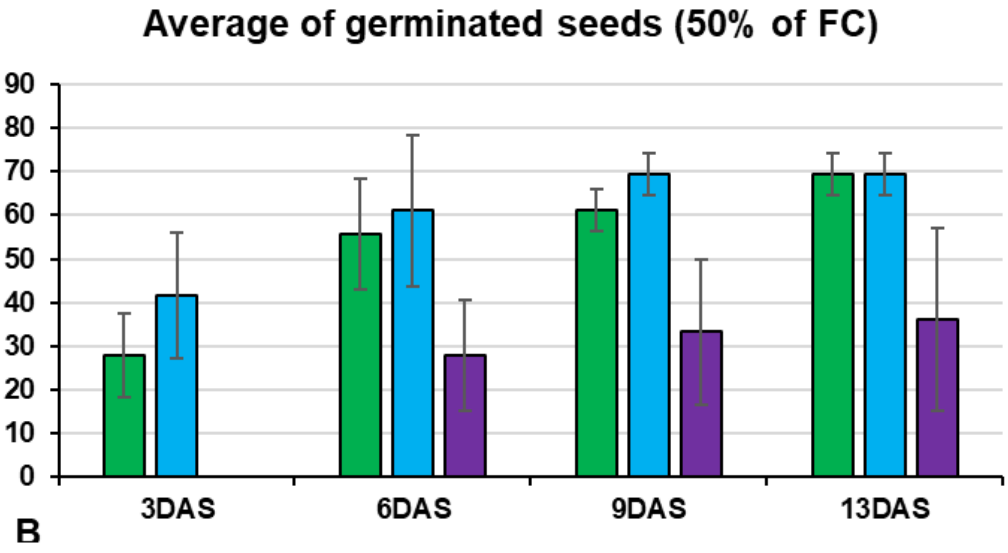
After the pre-sowing imbibition (see Figure 48) with water only (W) and diluted wood vinegar (WV), the trial germination started.

Figure 48. Safflower seeds (left) and pre-sowing seeds imbibition with water only (centre) and wood vinegar (right).

The test duration lasted 13 days, and overall, germination was negatively affected by the lowest water regime. Under severe water stress (25% of FC), the final germination rate was markedly reduced, reaching only about 65%, compared to approximately 70% at 75% FC. With this severe stress level, all treatments suffered, resulting in very low germination; in this case, even the water imbibition treatment proved ineffective at enhancing seed germinability. By contrast, a clear pattern emerged at the 75% and 50% FC regimes, where seeds imbibed with wood vinegar consistently showed the lowest germination, indicating an inhibitory effect of the solution on this specific variety. This inhibitory trend was evident from the beginning of the test and persisted throughout the monitoring period. Conversely, the control treatment and the simple water imbibition treatment resulted in similar and higher germination rates under both of these moderate water regimes.

Figure 49: Safflower seed in germination during the trial.





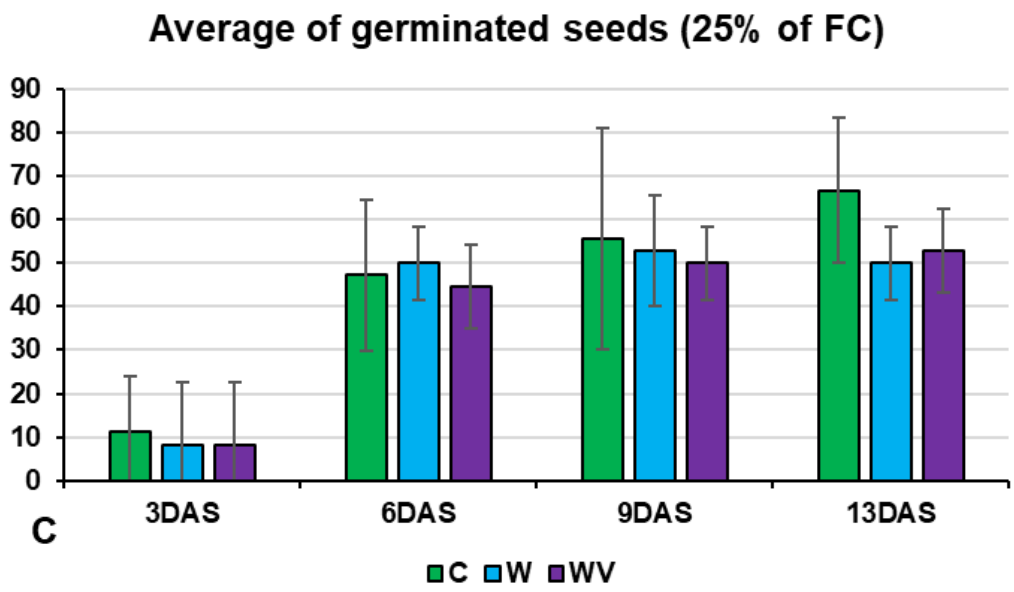


Figure 50. Average of safflower germinated seeds measured at 3, 6, 9 and 13 days after the sowing. In each histogram, the vertical bar represents the standard deviation.

6.7.2 Conclusions

Based on the germination trials, the three tested species demonstrated highly variable responses to the imbibition treatments and water regimes, underscoring the necessity of species-specific protocol optimization. The castor bean trial exhibited high variability and a low final germination rate overall (approximately 20%). However, it was the only species where imbibition, whether with distilled water or wood vinegar, improved germinability compared to the untreated control. **Interestingly, the wood vinegar imbibition treatment under water stress performed the best, also demonstrating the lowest variability, suggesting that the compounds within the wood vinegar may have enhanced seed vigor and conferred initial stress tolerance for this specific species.**
In contrast, sorghum exhibited a high overall germination rate (67% to 92%) regardless of treatment. For this species, the highest germination percentages were achieved at 75% and 50% of the field capacity. There were no significant differences observed among the three imbibition treatments, confirming that the wood vinegar solution had no inhibitory effect on sorghum germination.
Finally, the safflower demonstrated the most sensitivity to the wood vinegar solution. While all treatments suffered severely under the lowest water regime (25% FC), a clear inhibitory effect was observed at the moderate water regimes (75% and 50% FC), where seeds imbibed with wood vinegar consistently showed the lowest germination rates compared to both the control and water-imbibed seeds. **These contrasting results highlight that the effects of using pyrolysis-derived solutions are highly dependent on the plant species, ranging from beneficial (Castor Bean) to neutral (Sorghum) to clearly detrimental (Safflower).**

7. Conclusions

The activities described in this Deliverable D4.11 confirm the success of the operations planned in Task 4.2.4, demonstrating the technical feasibility of producing and valorizing biochar and wood vinegar (pyrolysis condensate) within the MIDAS project value chains. The investigations conducted, ranging from laboratory to demonstrative scale, provided a clear picture of the potential and limitations of the materials investigated.
In summary, the main results obtained are as follows:

- **Biochar production and quality:** the laboratory-scale carbonization campaign confirmed that most of the project's residual biomasses (including Guayule bagasse, Crambe, Miscanthus, and Yellow Melilot) are suitable for producing high-quality biochar. Characterization analyses highlighted the compliance of these materials with European regulations (EU FPR 2019/1009) and voluntary standards (EBC), with carbon stability parameters (H:C molar ratio and fixed carbon content) attesting to their effectiveness as long-term carbon sinks. Only *Safflower* hulls presented a Zinc content slightly above EBC limits for organic agriculture, although remaining promising for conventional farming. The demonstrative scale production successfully supplied the required quantities of woody biochar (120 kg on a dry basis) for field testing (Task 2.2.3), ensuring a safe material fully compliant with soil amendment standards.
- **Innovative applications:** the exploration of alternative biochar uses provided mixed but valuable indications. The use of micronized biochar in seed coating proved technically feasible. Preliminary germination tests on coated seeds highlighted a strong species dependence: while *Sorghum* and *Maize* showed no negative effects, *Common Bean* suffered reduced germinability, suggesting a specific sensitivity that may require replacing the synthetic binder (PEG) with biological alternatives like starch. Simultaneously, biochar was characterized and sent for evaluation as a bio-based substitute for Carbon Black in mulching films, opening up scenarios for industrial circular economy, although the grinding phase to reach the desired particle size requires high energy demand.
- **Wood vinegar production and characterization:** the production of wood vinegar was successfully carried out at a pilot scale using the SPYRO plant, processing poplar wood chips through a slow pyrolysis process at 500°C. The liquid products were effectively recovered and separated into bio-oil and an aqueous condensate phase (wood vinegar). The characterization of this aqueous fraction revealed a highly acidic profile (pH 2.77) and a high Total Organic Carbon content (65,719 mg/L). Detailed chemical analysis identified Acetic Acid as the primary component (95.99 mg/L), alongside significant concentrations of Methanol, Glycerol, Furfural, and phenolic compounds. These properties confirm the successful generation of a bioactive-rich condensate suitable for further agronomic valorisation as biostimulant, when diluted.
- **Wood vinegar valorization:** the characterization of the aqueous condensate (wood vinegar) identified acetic acid as the main bioactive compound (95.99 mg/L), establishing a clear dilution protocol (1 mM target) to mitigate acidity and avoid phytotoxic effects. Germination trials under water stress conditions revealed a highly variable species-specific response:
    - **Positive:** In *Castor Bean*, the treatment significantly improved germinability and reduced variability under water stress.

- **Neutral:** In *Sorghum*, no significant differences were observed compared to the control.
- **Negative:** In *Safflower*, wood vinegar imbibition reduced germination rates, indicating potential toxicity for this species at standard dosages.

The work conducted has provided project partners with the necessary materials (biochar and wood vinegar) and protocols for the large-scale agronomic trials (Task 2.2.3). The results underscore that, while the potential of these pyrolysis-derived products is high for both carbon sequestration and crop support, their application cannot be generalized. Instead, a "tailor-made" approach is essential, one that accurately selects the starting matrices based on pollutant content and adapts biostimulant dosages to the specific physiological tolerance of the target crops.

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