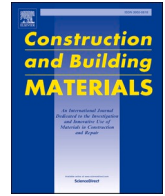




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Biological colonisation in bio-materials and composites with different bio-binder combinations

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ABSTRACT

The increasing demand for sustainable and biodegradable materials in the construction industry highlights the importance of developing circular bio-based building materials that align with the principles of circular bio-economy. This study forms part of a broader investigation aimed at developing novel construction materials with suitable thermal and acoustic performance. In this context, the present work focuses on evaluating the biological colonisation susceptibility of several natural organic materials in raw form and when used with bio-binder's, with particular attention to their fungal resistance in humid environments. The studied materials are rice husk, rice straw, *Posidonia oceanica* and sunflower stalk, alone and in combination with three different binders: arabic gum, xanthan gum and sodium alginate. Fungal resistance was tested exposing samples for four weeks under controlled temperature and relative humidity conditions, allowing quantification of mould growth. Results indicate that the tested unbound organic materials are highly susceptible to fungal colonisation. However, fungal proliferation could be significantly reduced by the addition of binders. Among the binders tested, sodium alginate showed the highest effectiveness in limiting fungal growth for all the tested materials, especially when combined with *Posidonia oceanica*, which presented the lowest bio-colonisation susceptibility. On the other hand, rice husk and sunflower stalk showed the highest fungal susceptibility, even with the application of binders. The results of this work contribute to a better selection of bio-materials and binders in order to enhance durability and sustainability for bio-based construction composites, contributing to the implementation of circular economy strategies in the built environment.

1. Introduction

The drive towards sustainability in the construction industry, prompted by the climate emergency and framed within the European Union's strategy for a circular bioeconomy, is accelerating the development of circular bio-based building materials [1,2]. These materials align with the Sustainable Development Goals outlined in the 2030 Agenda [3] shared by the European Union and the United Nations, have encouraged the use of bio-based materials as a sustainable substitute for conventional building materials. Derived from renewable biomass such as agricultural residues, plant fibres and agro-industrial waste, these

circular bio-based materials offer significant advantages in reducing the environmental footprint of the built environment [4,5]. Among their key benefits, bio-based materials have a reduced environmental impact, owing to lower energy use in their development and carbon usage, making them highly suitable for sustainable construction applications [6]. In this context, the use of natural binders—such as clay and lime—represents a promising strategy for maintaining the sustainability of bio-based composites. Although clay and lime have traditionally been used in construction, they have mostly served as primary materials, often reinforced with natural fibres—rather than as binders within lightweight composites [7–9]. Their physical properties, such as high

Abbreviations: AS, Sodium Alginate; GA, Arabic Gum; GX, Xanthan Gum; RH, Rice Husk; RS, Rice Straw; SS, Sunflower Stalk; PO, *Posidonia oceanica*.

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density and rigidity, make them less suitable for the development of materials specifically intended for thermal insulation and acoustic conditioning. For this reason, alternative binders are being explored to meet the specific requirements of these applications. In particular, polysaccharides such as arabic gum, xanthan gum and sodium alginate, which are explored in this study, offer novel possibilities for enhancing the performance and environmental profile of bio-based materials [10–13]. In some studies, investigating different natural residues combined with bio-binders, key approaches have been explored to understand how the intrinsic properties of materials—such as fibrous structure, chemical composition and water interaction—affect their overall performance [14–16].

One of the main concerns regarding bio-based materials is that they can be susceptible to fungal growth, which compromise in terms of durability and long-term performance under fluctuating environmental conditions [17,18]. Several studies have assessed the fungal susceptibility of bio-based composites manufactured with traditional natural binders such as earth and lime [19–21]. These investigations consistently highlight that higher organic fibre content generally correlates with increased fungal colonisation, mainly due to elevated water retention and nutrient availability. Such findings underscore the importance of considering both the binder and the aggregate when evaluating the biotic durability of these materials. The presence of relative moisture combined with favourable temperature conditions create a fungal growth environment leading to material degradation and health risk through indoor air pollution [22,23]. Such material vulnerability is determined by composition, density, porosity and water holding capacity, which may affect the colonisation by fungal species [24]. Given these risks, extensive fungal susceptibility testing has been emphasised, particularly for insulation materials [25] where moisture retention plays a critical role in maintaining long-term performance [26]. Hoang et al. [26] conducted research on the resistance of different green building materials to fungal growth, showing considerable variability in susceptibility based on material composition. The results demonstrate that some bio-based materials are naturally resistant to fungal colonisation, whereas other materials need to be equipped with extra treatments to avoid microbial deterioration, consequently emphasising the importance of using some protection measures. Comparative studies have further examined the efficiency of several different experimental methodologies for assessing fungal susceptibility, facilitating the development of criteria for standardised assessments [27]. While bio-based materials are gaining traction in the construction industry, it is vital to conduct bio-susceptibility studies to assess their viability as a building material and composites.

Organic natural binders, such as sodium alginate and arabic and xanthan gum, are obtained from renewable sources, are non-toxic, have low density and low thermal conductivity and are biodegradable. Regarding their behaviour in front of biotic attacks, the antifungal activity of xanthan gum, arabic gum and sodium alginate has been studied [28–30] and several studies have reported the use of these binders as carriers for antifungal agents [31]. In the case of sodium alginate, Rahman A.M. et al. described that the addition of sodium alginate to potato dextrose agar reduced fungal growth [32]. In this work, the influence of different bio-based aggregates as well as the combination with different natural organic binders on fungal growth has been evaluated.

This study aims to evaluate the resistance to fungal growth of various bio-based building materials, both in their raw form and as part of composites that contain a natural organic binder. These composites should be especially suited for use as thermal insulation panels or boards for internal partitions or as infill in wall assemblies, where moisture conditions are moderate and low environmental impact is desired. Following established testing guidelines, the research aims to provide insights into the development of durable, eco-efficient and bio-resistant building composites, contributing to advancements in sustainable construction and its circularity.

2. Materials and methodology

2.1. Materials and composites

The western regions of Catalonia, specifically Lleida and Tarragona, are characterised by significant agricultural activity, which leads to the generation of substantial amounts of plant waste. In addition, the province of Tarragona also obtains other types of plant waste, located in its coastal areas. These factors motivated the decision to use various plant wastes for this study on bio-construction materials and bio-based insulation composites. After an initial selection of more than 12 materials, which were subjected to an initial physical and chemical study, performed by this team [33], four were finally selected based on their availability and characteristics. The selected materials are:

- Rice straw (RS) and husk (RH), due to the significant rice production in the Ebro Delta.
- Sunflower stalk (SS), which are produced in abundance in the province of Lleida.
- *Posidonia oceanica* (L.) Delile (PO), a Mediterranean marine plant that accumulates on the beaches of the *Costa Daurada* after its natural decomposition and which must be removed by the local councils owing to the large quantities accumulated and in order to obtain the blue flag quality label for the beaches in the area (Fig. 1).

In addition to availability, the selection considered the distinct characteristics of each material. This diversity allowed the exploration of different structural and compositional properties. The external and internal structures of these materials present significant differences, further enriching the research potential for the development of bio-based construction composites.

The natural binders were selected with the aim of preserving the environmental integrity of the developed composites. Preliminary tests assessed the binding potential of rice starch, wheat starch, Arabic gum, and xanthan gum. Starch-based binders were deemed unsuitable owing to their high dosage requirements with a water-to-binder ratio of 1:1. Additionally, their inefficiency became evident as, although the binder initially appeared to successfully adhere the fibres, the composite structures, particularly those composed of particles such as rice husk and sunflower stalk, later exhibited significant disintegration. This instability made the compounds difficult to handle and limited their practical application. Consequently, starch-based binders were excluded from this study. In contrast, arabic gum and xanthan gum demonstrated superior binding efficiency and structural stability, making them the focus of further optimisation. In addition, a third binder of a different typology was also used: sodium alginate, as it had been used in several studies on sunflower samples and showed promising results [16].

The optimisation of the binder formulation was carried out through a practical trial-and-error approach. The main criteria considered during this process was the workability of the fresh mixture and the cohesion of the composite after drying. Various water-to-binder and binder-to-material ratios were tested to identify the minimum binder content required to achieve sufficient structural integrity and ease of application. The aim was to ensure adequate adhesion and handling while preserving the porosity and lightweight character of the composites. Although literature was consulted to inform the range of initial proportions tested, the final values were determined empirically, as specific guidelines for natural binder applications in bio-based insulation composites remain limited. Only the ratios providing good cohesion without compromising porosity or leading to disintegration were selected for final testing. For arabic gum, an optimal ratio of 2:1 was established, while xanthan gum required a higher dilution, with a ratio of 40:1. The preparation of sodium alginate matrix requires a more elaborate process compared to other binders to obtain a stable gel. In addition to water, the method involves the addition of a source of Ca^{2+} cations to promote the gelation process. Two common sources of calcium are gypsum stone



Fig. 1. Studied raw materials: from left to right rice husk (RH), rice straw (RS), sunflower stalk (SS) and *Posidonia oceanica* (PO).

and sodium citrate. Initially, sodium alginate is dissolved in water in a water-to-binder ratio of 65.7:1. This solution is allowed to stand for 24 h to achieve homogeneity. Once the solution is ready, it is combined with gypsum and citrate solutions in mass proportions of 1:0.15 in both cases, just before its application as a binder agent. The proportions for the additional components are as follows: gypsum-to-water: 1:36.9 and citrate-to-water: 1:22.3.

The binder:material mass proportions are synthesised in Table 1 below.

The variability of the binding behaviour between the materials required customised adjustments in the formulations. This systematic approach enabled the preparation of consistent and reproducible composite samples, which is essential for the accurate evaluation of their properties.

According to the test protocol, four samples measuring 40 mm × 40 mm × 40 mm were prepared for each material and composite combination, resulting in a total of 48 bonded samples.

2.2. Methodology

The method used to evaluate the biological susceptibility of the materials and composites was adapted from ASTM D5590–17 standard [34].

Prior to testing, all samples were sterilised according to the standard protocol, using an autoclave at 100°C for 20 min and past 24 h another autoclave cycle is performed for 10 min this time. This sterilisation process was applied to all prepared samples, including the raw materials. Subsequently, two different preparation methods were used depending on whether the samples were raw materials or composites.

For the raw materials, four 9 cm Petri dishes were prepared for each sample type, resulting in a total of sixteen plates. For the composite materials, four cylindrical containers were used for each material–binder combination, totalling forty-eight containers (see Fig. 2). Each Petri dish was filled with 20mL and each container with 60 mL of a culture medium composed of 4 % malt and 2 % agar. Prior to placing the samples, a plastic-coated metal mesh was positioned between the

Table 1

Binder to material ratios used in the present study, among the materials studied, rice husk, rice straw, sunflower stalk and *P. oceanica* and the agglutinates arabic gum, xanthan gum and sodium alginate.

Material	Binder		
	Arabic Gum (GA)	Xanthan Gum (GX)	Sodium Alginate (AS)
Rice Husk (RH)	1:1.50	1:1.88	1:3.63
Rice Straw (RS)	1:1.50	1:3.33	1:3.63
Sunflower Stalk (SS)	1:1.50	1:2	1:3.63
<i>Posidonia oceanica</i> (PO)	1:1.25	1:1	1:3.63

material and the surface of the solidified medium in order to avoid direct contact. This configuration allowed for fungal exposure under controlled conditions while preventing submersion or direct material–substrate interaction, ensuring a representative simulation of surface contamination.

The moulds *Aspergillus niger* Tiegh. and *Penicillium funiculosum* (De Bary) G. Arnaud were used, according with the standard used and also owing to their high prevalence in indoor environments. The fungal strains used for inoculation were obtained from the fungal culture collection at LNEC (National Laboratory for Civil Engineering, Lisbon, Portugal). A suspension containing a mixture of spores was prepared and 1 mL of this suspension was inoculated into each Petri dish and container already containing the materials to be analyse.

Once all containers with the materials and culture medium were prepared, they were placed in a climate chamber for four weeks under controlled conditions of 22°C ± 1°C temperature, and 70 % ± 5 % relative humidity. Visual inspections of the samples were performed weekly and mould growth was evaluated according to the classification criteria shown in the Table 2.

The assessment of mould-covered surface area on each sample was performed through direct visual inspection, following the criteria defined in ASTM D5590–17 [34]. The extent of fungal growth was estimated by visually comparing the area covered with mould to the total surface of each sample. Based on this estimation, each sample was assigned to a contamination category from 0 to 4, as shown in Table 2. In ambiguous cases, stereomicroscopic observation was used to confirm fungal presence.

At the end of the four-week period, the samples were subjected to a final visual assessment and examined with a stereomicroscope (Olympus SZX12) to observe mould growth at higher magnifications and confirm the visual observations.

Following visual and microscopic observation, two-way analysis of variance (ANOVA) was used to examine the individual and joint effects of the two factor variables—binder type and material type—on mould growth. This statistical method examines differences in mean fungal growth among groups and interactions between the variables. Post-ANOVA, Tukey's Honestly Significant Difference (HSD) post-hoc test was used to determine pairwise differences among the group means. The tests were selected based on their precision for evaluating multi-factorial experimental designs and their capability for maintaining type I error for multiple comparisons. Statistical analyses were performed using Python (v3.13.2) on macOS, employing the statsmodels and scipy libraries. For all statistical evaluations, a significance level of $p < 0.05$ was chosen.

3. Results and discussion

The results of this test exhibited significant variation depending on both the material and the type of binder used. A detailed analysis of each material is provided in the following sections, after which Table 3 will

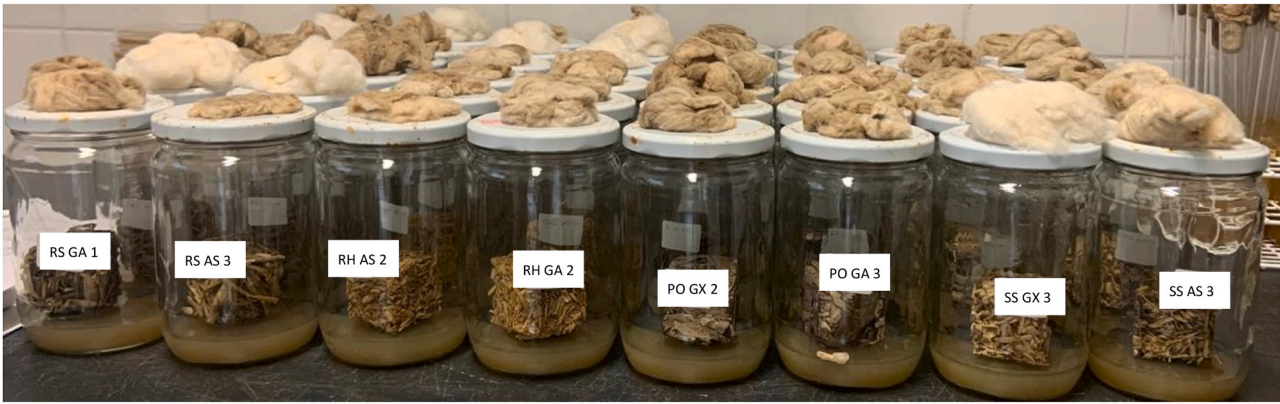


Fig. 2. Composite samples placed in containers prior to their introduction into the climate chamber to begin the bio-susceptibility test. The combinations of the composite samples are of the raw materials rice husk (RH) rice straw (RS), sunflower stalk (SS) and *Posidonia oceanica* (PO) with arabic and xanthan gums and sodium alginate binders.

Table 2
Classification of mould development as defined in ASTM D5590-17 [34].

Rating	Description	Contaminated surface (%)
0	None	0 %
1	Traces of growth	< 10 %
2	Light growth	10–30 %
3	Moderate growth	30–60 %
4	Heavy growth	> 60 %

Table 3
Average rate (\pm standard deviation) of mould growth for all the samples, Rice Husk, Rice Straw, Sunflower Stalk and *Posidonia oceanica*, with (composites) and without binder (raw materials).

Material	Binder	Mould Development			
		Week 1	Week 2	Week 3	Week 4
Rice Husk	No Binder	4.00	4.00	4.00	4.00
	GA	2.50 (± 0.50)	4.00	4.00	4.00
	GX	2.00	3.00	3.00	3.00
	AS	1.00 (± 0.71)	1.50 (± 0.50)	2.00 (± 1.00)	2.50 (± 0.87)
	AS	2.75 (± 0.43)	4.00	4.00	4.00
Rice Straw	No Binder	2.75 (± 0.43)	4.00	4.00	4.00
	GA	1.25 (± 0.43)	1.25 (± 0.43)	2.33 (± 0.47)	3.00 (± 0.71)
	GX	1.00	2.00	2.67 (± 0.47)	3.33 (± 0.94)
	AS	0.75 (± 0.43)	1.00	1.33 (± 0.47)	2.75 (± 0.43)
	AS	4.00	4.00	4.00	4.00
Sunflower Stalk	No Binder	4.00	4.00	4.00	4.00
	GA	2.25 (± 0.43)	4.00	4.00	4.00
	GX	3.25 (± 0.43)	4.00	4.00	4.00
	AS	0.50 (± 0.50)	1.00	1.25 (± 0.43)	2.75 (± 0.83)
<i>Posidonia oceanica</i>	No Binder	4.00	4.00	4.00	4.00
	GA	0.00	0.00	0.00	0.75 (± 0.43)
	GX	0.00	0.50 (± 0.5)	0.50 (± 0.5)	1.00
	AS	0.00	0.00	0.00	0.50 (± 0.5)

present a comprehensive summary of fungal growth across all material-binder combinations for each week. Despite these variations, a consistent pattern was observed for the unbonded materials. In all cases, regardless of the material type, samples displayed substantial mould growth within the first week following fungal inoculation, covering between 30 % and 60 % of the surface, as shown in Table 2. By the second week, all unbonded materials reached the highest level of mould proliferation, classified as Heavy growth (more than 60 % of the surface covered with mould).

Fig. 3 illustrates the mould development observed in the first week for one specimen of each material. The image reveals widespread fungal distribution across the samples, with the exception of the RS material, which still shows areas of comparatively lower growth.

For RH samples, those bonded with GA developed mould covering more than 60 % of the surface by the second week, with the fungal growth increasing steadily up to the fourth week, eventually covering the entire surface and leading to a higher mould density and thicker fungal layers. Samples bonded with GX had widespread fungal colonisation, but the growth was weak compared with that achieved with GA. The binder with the best performance towards mould growth was AS: while mould growth occurred, it never covered the surface completely, reaching only about 30 % on average. Fig. 4 shows a sample from each of the cases after exposure for four weeks. For the first and third cases, there are quite a few scattered colonies of black spores that have formed all over the sample; The first case exhibited between 30 % and 60 % surface coverage, whereas the third case showed a lesser impact, with coverage not exceeding 30 %. The second sample was almost entirely covered by a thin, filamentous, web-like layer, displaying extensive growth of over 60 % according to Table 2. Additionally, it featured the same black spores observed in the other samples.

For the RS samples, the mould growth was considerably lower, and at the end of the four-week exposure period, it was observed that there was no full fungal coverage of the surface, affecting between 30 % and 60 %, in contrast to the more than 60 % observed in the other materials. The differences between the binders were relatively small; GA and GX had almost identical performances. AS performed slightly better with light growth, between the 10–30 % of surface covered, compared to the other binders, indicating its improved resistance compared to the other materials. Fig. 5 represents samples of each binder after four weeks. All samples show the growth of fungi on the top surface of the samples. Upon closer examination, mould development is more pronounced in the first and second cases. In the GX sample, shown in the first image, fungal growth with a greenish hue can be seen, in addition to the black spore formation seen in the other samples. In contrast, the third case shows fungal growth restricted to specific areas.

The SS exhibited the poorest performance among all tested materials.



Fig. 3. Studied raw materials in the Petri dishes, before and after one week of the inoculation: top the initial samples and bottom after one week and from left to right, rice husk (RH), rice straw (RS), sunflower stalk (SS) and *Posidonia oceanica* (PO).



Fig. 4. Composites of rice husk (RH) with the different binders: from left to right, xanthan gum (GX), arabic gum (GA) and sodium alginate (AS).



Fig. 5. Composites of rice straw (RS) with the different binders: from left to right, xanthan gum (GX), arabic gum (GA) and sodium alginate (AS).

By the second week, samples bonded with GA and GX displayed complete fungal coverage across their surfaces. In contrast, samples bonded with AS demonstrated a performance comparable to RH and RS, with significant mould growth that did not exceed 60 % of the surface in most cases. Fig. 6 illustrates the nearly identical behaviour observed in the first two cases using GX and GH as binders, with a full coverage by moulds, while the AS shows a markedly different pattern, with moulds covering around 30 % of the surface of the samples.

Finally, PO exhibited the best performance among all tested materials. Mould growth was visible only in samples bonded with GX and

even then, it covered less than 10 % of the surface. For samples bonded with AS and GA, fungal growth was observed only in isolated spots, detectable exclusively through microscopic examination, as no visible mould propagation was evident, having an average of less than 10 % of the surface contaminated, according to the ASTM-D5590-17 table, shown above in Table 2.

In the samples shown in Fig. 7, fungal growth is not easily detectable visually.

However, Fig. 8 presents magnified images obtained through microscopic observation, where small fungal colonies can be identified



Fig. 6. Composites of sunflower stalk (SS) with the different binders: from left to right, xanthan gum (GX), arabic gum (GA) and sodium alginate (AS).



Fig. 7. Composites of *Posidonia oceanica* (PO) with the different binders: from left to right, xanthan gum (GX), arabic gum (GA) and sodium alginate (AS).

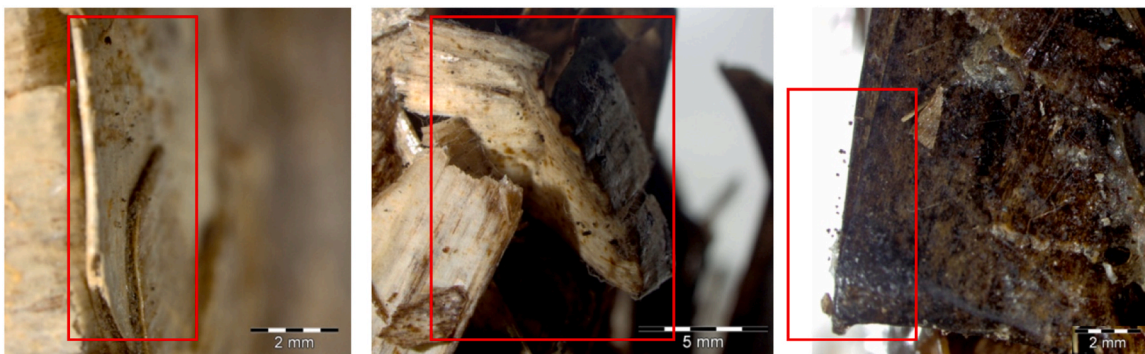


Fig. 8. Microscope images with different magnifications, indicated on the scale, of *Posidonia oceanica* (PO) composite samples with different binders: from left to right, xanthan gum (GX), arabic gum (GA) and sodium alginate (AS).

in PO composites with the different binders. In all images, the red box highlights the presence of small black spores on the samples. The primary difference between the binders in this case is that mould growth was detected in all GX-bonded samples, whereas in GA-bonded samples, mould growth was detected in three out of four specimens and in AS-bonded samples, only two out of four specimens showed mould presence.

To provide a more comprehensive overview of the results and facilitate comparisons between different materials and binders, Table 3 presents a summary of the average mould growth rates. These values are based on the ASTM D5590–17 standard [34] and were calculated as the average growth rate, according to Table 1, across all tested samples, along with their respective standard deviations.

The results presented in Table 3 indicate that raw materials, without any binder, exhibit a very high biological susceptibility, while the presence of binders on the composites helps to in reduce fungal growth to some extent. Among the composites, SS showed the highest susceptibility to mould development, followed by RH, whereas PO demonstrated the lowest biological susceptibility when combined with a binder.

Regarding the effectiveness of the binders, AS was the most effective in inhibiting mould growth. Across all materials, samples agglomerated with AS consistently exhibited the lowest percentage of mould growth, with the PO-AS combination showing the best performance.

To validate these observations statistically, a two-way analysis of variance (ANOVA) was conducted to evaluate the influence of both material type and binder on mould growth. The analysis revealed a significant effect of both factors ($F = 69.47$, $p < 0.001$ for material and $F = 38.02$, $p < 0.001$ for binder), as well as a significant interaction between them ($F = 8.94$, $p < 0.001$), indicating that the impact of the material varied depending on the binder.

To further understand the differences in mould growth across the various combinations of materials and binders, a Tukey's post-hoc test was performed following the two-way ANOVA. The analysis showed significant differences between the various combinations of materials and binders and hence improved the understanding of both the interactions and individual effects of the materials and binders on mould growth.

Significant differences in fungal susceptibility were observed between *Posidonia oceanica* composites (PO_AS, PO_GA, PO_GX) and all

other materials ($p = 0.0$). Within the rice husk group, RH_AS also differed from RH_GA and RH_NoBinder ($p = 0.0362$). Similarly, rice straw composites RS_AS showed significant differences with RS_NoBinder ($p = 0.0$). Finally, sunflower stalk (SS) samples differed significantly with both RH_AS and RS_AS ($p = 0.0362$).

These results are displayed graphically in Fig. 9 where the samples are grouped into statistically significant categories denoted by the letters a, b and c. Samples sharing the same letter do not exhibit significant differences, whereas those labelled with different letters show statistically significant differences.

These findings suggest that unbound materials do not show significant differences among themselves, so material use alone does not influence mould growth. In contrast, PO exhibited consistent significant differences with RH, RS and SS materials across all binder types, indicating a strong influence of the material-binder combination on mould growth. Furthermore, RH_AS and RS_AS showed significant improvements compared to their unbound counterparts, highlighting the effectiveness of AS in reducing mould susceptibility.

This analysis supports the findings observed in Table 3, where AS was the most effective binder at inhibiting mould growth. Among all materials, samples bonded with AS consistently showed the lowest percentage of mould growth, with the PO-AS combination showing the best performance. Selecting an appropriate binder, such as AS, can significantly influence the mould susceptibility of organic materials-based composites, potentially improving their durability and resistance to biodegradation in humid environments.

4. Conclusions

The experimental results obtained within this research study have allowed estimating the bio-susceptibility of different organic materials, in unbound forms and as lightweight building composites, combined with various organic natural binders, to the growth of fungi.

Unbound natural materials showed a high susceptibility to fungal

growth: extensive mould proliferation was recorded on all types of materials from the first week of exposure, reaching its peak in the second week. The absence of a binder leaves the organic matrix exposed, facilitating fungal proliferation. These results underscore the necessity of incorporating protective strategies, such as binder addition, in bio-based composites intended for humid environments.

The combination with an organic natural binder significantly reduced mould growth, although its effect depended on the type of biomaterial used and the type of binder. Sunflower stalk was found to be the biomaterial most susceptible to fungal growth, with full colonisation by the second week, while *Posidonia oceanica* showed the greatest resistance to mould growth, especially when combined with sodium alginate. These findings highlight the importance of the intrinsic properties of each material in determining susceptibility, beyond the effect of the binder alone.

Among the binders tested, sodium alginate provided the best biologically resistant across all composites with the test materials, performing as a partial physical barrier against the fungal development. Arabic gum and xanthan gum were less effective, particularly when combined with rice husk and sunflower stalk, where contamination levels remained high. The *Posidonia oceanica* and sodium alginate combination was the most successful overall, having very little sign of fungal activity even when viewed microscopically. This calls for careful selection of adequate bio-binder-biomaterial combinations to develop durable bio-based insulation composites.

These results put into evidence that the nature of bio-material, as well as the type of binder, has to be considered in the development of lightweight building insulation composites with improved resistance to fungi growth to be applied where durability and biological stability are critical factors.

While the results presented provide valuable insight into the biological susceptibility of bio-based composites, this study has certain limitations. As a first point, the duration of exposure was only four weeks and, in so doing and although under harsh conditions, might not

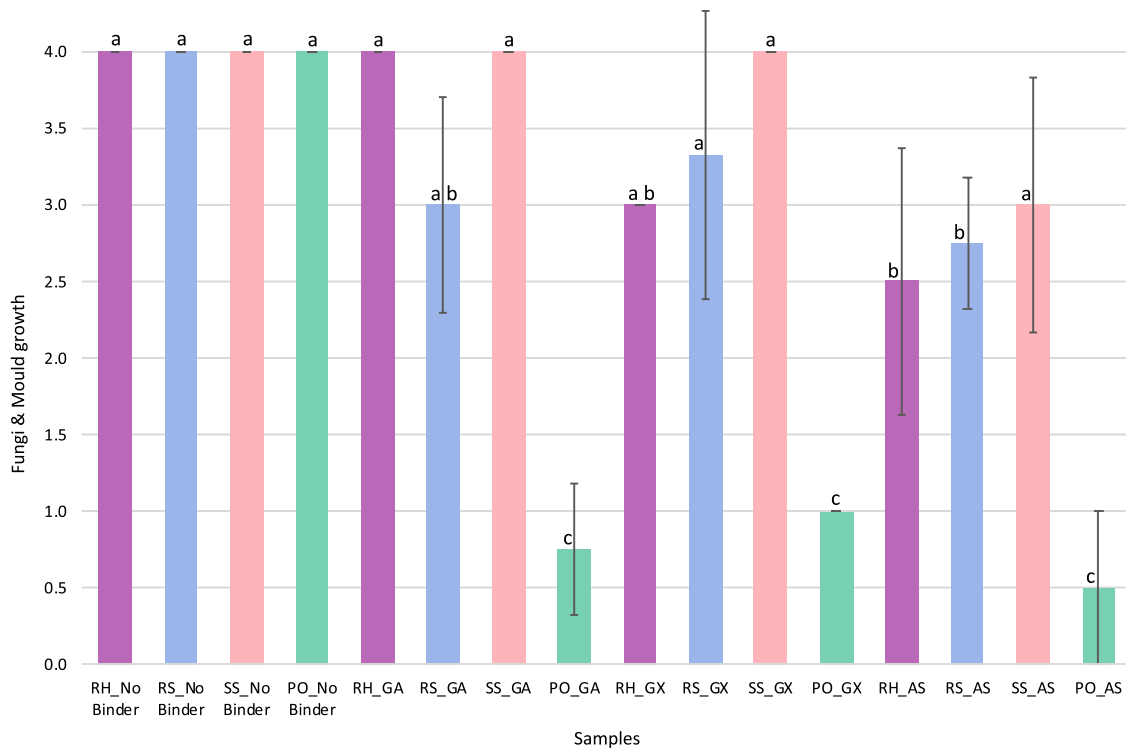


Fig. 9. Bar chart showing the mean growth after four weeks for the combinations of different materials and binders, including standard deviation. Significant differences between samples are indicated by the letters a, b and c. Samples sharing the same letter do not show significant differences, while samples with different letters exhibit statistically significant differences. Bars of the same colours correspond to the same fibres.

adequately depict extended fungal colonisation. Second, the tests were done in laboratory conditions where temperature and humidity were regulated, without considering the overall variability provided by environmental conditions like season, ventilation, or microbial diversity in various geographic areas. Future research should consider extending the time of exposure of fungi to observe long-term biodegradation. Also, conducting tests in dynamic or semi-natural environmental systems would enhance the understanding of the material's and composites performance in realistic environments. Future research can investigate more varied combinations of binder blends and explore natural additives that have promising antifungal properties to achieve improved durability along with sustainability.

CRedit authorship contribution statement

Paulina Faria: Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization. **Sónia Duarte:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization. **Lacasta Ana:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Laia Haurie:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Marta Duarte:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization. **Brenda Arias-Cárdenas:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

- [1] D. Jablonowski, M. Von Cossel, Y. Iqbal, M.C.M. Parlato, A. Pezzuolo, From field to building: harnessing Bio-Based building materials for a circular bioeconomy, *Agronomy* 14 (9) (Sep. 2024) 2152, <https://doi.org/10.3390/AGRONOMY14092152>.
- [2] D.L. Le, R. Salomone, Q.T. Nguyen, Circular bio-based building materials: a literature review of case studies and sustainability assessment methods, *Build. Environ.* 244 (Oct. 2023) 110774, <https://doi.org/10.1016/J.BUILDENV.2023.110774>.
- [3] UN General Assembly, Transforming our world: the 2030 Agenda for Sustainable Development, A/RES/70/1, 21 October 2015, Accessed: Mar. 17, 2025. [Online]. Available: (<https://www.refworld.org/legal/resolution/unga/2015/en/111816>).
- [4] F. Asdrubali, F. D'Alessandro, S. Schiavoni, A review of unconventional sustainable building insulation materials, *Sustain. Mater. Technol.* 4 (Jul. 2015) 1–17, <https://doi.org/10.1016/j.susmat.2015.05.002>.
- [5] E. Cintura, P. Faria, M. Duarte, L. Nunes, Bio-Wastes as aggregates for Eco-Efficient boards and panels: screening tests of physical properties and Bio-Susceptibility, *Infrastructures* 7 (3) (2022), <https://doi.org/10.3390/infrastructures7030026>.
- [6] L.C. De Ligne, J.B.B. Van Den Bulcke, J.M. Baetens, B. De Baets, J.C.M. Van Acker, Bio-Based building materials - how to unravel the role of material characteristics on fungal susceptibility? *Curr. Top. Trends Durab. Build. Mater. Compon. Proc.* 15th Int. Conf. Durab. Build. Mater. Compon. DBMC 2020 (Jan. 2020) 239–246, <https://doi.org/10.23967/dbmc.2020.195>.
- [7] G. Di Bella, V. Fiore, G. Galtieri, C. Borsellino, A. Valenza, Effects of natural fibres reinforcement in lime plasters (kenaf and sisal vs. Polypropylene), *Constr. Build. Mater.* 58 (May 2014) 159–165, <https://doi.org/10.1016/J.CONBUILDMAT.2014.02.026>.
- [8] G. Giuffrida, L. Ibos, A. Boudenne, H. Allam, Analysis of the thermal performances of uninsulated and bio-based insulated compressed earth blocks walls: from the material to the wall scale, *J. Build. Eng.* 90 (2024) 109370, <https://doi.org/10.1016/J.JOBE.2024.109370>.
- [9] S. Paul, M.S. Islam, T.E. Elahi, Comparative effectiveness of fibers in enhancing engineering properties of earth as a building material: a review, *Constr. Build. Mater.* 332 (2022) 127366, <https://doi.org/10.1016/J.CONBUILDMAT.2022.127366>.
- [10] M. Nasir, A.B.M.S. Islam, K.S. Alotaibi, W. Al-Kutti, Evolution of arabic Gum-based Green mortar towards enhancing the engineering properties – fresh, mechanical, and microstructural investigation, *Constr. Build. Mater.* 365 (2023) 130025, <https://doi.org/10.1016/J.CONBUILDMAT.2022.130025>.
- [11] N. Prasad, N. Thombare, S.C. Sharma, S. Kumar, Gum arabic – a versatile natural gum: a review on production, processing, properties and applications, *Ind. Crops Prod.* 187 (2022) 115304, <https://doi.org/10.1016/J.INDCROP.2022.115304>.
- [12] S.H. Imam, C. Bilbao-Sainz, B.Sen Chiou, G.M. Glenn, W.J. Orts, Biobased adhesives, gums, emulsions, and binders: current trends and future prospects, *J. Adhes. Sci. Technol.* 27 (18–19) (2013) 1972–1997, <https://doi.org/10.1080/01694243.2012.696892>.
- [13] R.V. Gadhave, R.V. Gadhave, Xanthan Gum—Bio-Based raw material for wood adhesive, *Green. Sustain. Chem.* 13 (2) (2023) 153–161, <https://doi.org/10.4236/GSC.2023.132008>.
- [14] E. Cintura, P. Faria, L. Molari, L. Barbaresi, D. D'Orazio, L. Nunes, Characterization of an arundo donax-based composite: a solution to improve indoor comfort, *Ind. Crops Prod.* 208 (2024) 117756, <https://doi.org/10.1016/j.indcrop.2023.117756>.
- [15] E. Cintura, L. Nunes, P. Faria, A review of laboratory tests to evaluate Agro-Industrial wastes properties as building materials, in: *RILEM Bookseries*, 41, Springer Science and Business Media B.V., 2023, pp. 55–66, https://doi.org/10.1007/978-3-031-29191-3_5.
- [16] M. Palumbo, Contribution to the development of new bio-based thermal insulation materials made from vegetal pith and natural binders: hygrothermal performance, fire reaction and mould growth resistance. *Architecture and Building Construction, Universitat Politècnica de Catalunya (UPC), Barcelona, Ph.D. dissertation, Dept. of Technology, Spain, 2015.*
- [17] E. Cintura, L. Nunes, B. Esteves, P. Faria, Agro-industrial wastes as building insulation materials: a review and challenges for Euro-Mediterranean countries (Art. no), *J. Build. Eng.* 66 (2023) 105909, <https://doi.org/10.1016/j.jobe.2022.105909>.
- [18] B. Andersen, T.V. Rasmussen, Biobased building materials: moisture characteristics and fungal susceptibility, *Build. Environ.* 275 (May 2025) 112720, <https://doi.org/10.1016/J.BUILDENV.2025.112720>.
- [19] K. Chau, R. Fleck, P.J. Irga, F.R. Torpy, S.J. Wilkinson, A. Castel, Hempcrete as a substrate for fungal growth under high humidity and variable temperature conditions, *Constr. Build. Mater.* 398 (Sep. 2023) 132373, <https://doi.org/10.1016/J.CONBUILDMAT.2023.132373>.
- [20] A. Laborel-Préneron, et al., Laboratory test to assess sensitivity of bio-based earth materials to fungal growth, *Build. Environ.* 142 (Sep. 2018) 11–21, <https://doi.org/10.1016/J.BUILDENV.2018.06.003>.
- [21] A. Simons, A. Bertron, C. Roux, A. Laborel-préneron, J.E. Aubert, C. Roques, Susceptibility of earth-based construction materials to fungal proliferation: laboratory and in situ assessment, *RILEM Tech. Lett.* 3 (Jul. 2018) 140–149, <https://doi.org/10.21809/RILEMTECHLETT.2018.69>.
- [22] K. Coombs, S. Vesper, B.J. Green, M. Yermakov, T. Reponen, Fungal microbiomes associated with Green and Non-Green building materials, *Int. Biodeterior. Biodegrad.* 125 (0) (2017) 251, <https://doi.org/10.1016/J.IBIOD.2017.07.018>.
- [23] L. Deborde, Y. Andres, C. Lanos, F. Collet, Assessment of fungal development risk on bio-based thermal insulation, *Ind. Crops Prod.* 222 (2024) 119889, <https://doi.org/10.1016/J.INDCROP.2024.119889>.
- [24] K. Gradedi, N. Labonnote, B. Time, J. Köhler, Mould growth criteria and design avoidance approaches in wood-based materials – a systematic review, *Constr. Build. Mater.* 150 (2017) 77–88, <https://doi.org/10.1016/J.CONBUILDMAT.2017.05.204>.
- [25] L. Nunes, S. Duarte, J.L. Parracha, D. Jones, I. Paulmier, M. Kutnik, Insulation materials susceptibility to biological degradation agents: molds and subterranean termites, *Appl. Sci.* 13 (20) (2023), <https://doi.org/10.3390/app132011311>.
- [26] C.P. Hoang, K.A. Kinney, R.L. Corsi, P.J. Szanislo, Resistance of Green building materials to fungal growth, *Int. Biodeterior. Biodegrad.* 64 (2) (2010) 104–113, <https://doi.org/10.1016/J.IBIOD.2009.11.001>.
- [27] T. Verdier, M. Coutand, A. Bertron, C. Roques, A review of indoor microbial growth across building materials and sampling and analysis methods, *Build. Environ.* 80 (2014) 136–149, <https://doi.org/10.1016/J.BUILDENV.2014.05.030>.
- [28] A. Tøndervik, et al., Alginate oligosaccharides inhibit fungal cell growth and potentiate the activity of antifungals against candida and aspergillus spp, *PLoS One* 9 (11) (2014), <https://doi.org/10.1371/JOURNAL.PONE.0112518>.

- [29] M. Snoussi, M. Najett, A.S. Djamel, Evaluation in vivo antifungal effect of gum arabic of acacia tortilis (Forssk) on storage deteriorating fungi by coating method, *Res. J. Pharm. Technol.* 13 (12) (2020) 5668–5672, <https://doi.org/10.5958/0974-360X.2020.00987.7>.
- [30] N. Hajar, et al., Antibacterial and antifungal properties of cassava starch, xanthan gum and zinc oxide nanoparticles coating solution for banana (*Musa acuminata*) preservation, *BIO Web Conf.* 165 (2025), <https://doi.org/10.1051/BIOCONF/202516501002>.
- [31] C. de, C. Spadari, L.B. Lopes, K. Ishida, Potential use of Alginate-Based carriers as antifungal delivery system, *Front. Microbiol.* 8 (2017) 97, <https://doi.org/10.3389/FMICB.2017.00097>.
- [32] A.M. Rahman, et al., Effects of sodium alginate and calcium chloride on fungal growth and viability in Biomass-Fungi composite materials used for 3D printing, *Biomim.* Vol. 9 Page 251 9 (4) (2024) 251, <https://doi.org/10.3390/BIOMIMETICS9040251>.
- [33] B.A. Cárdenas, A.M. Lacasta, L. Haurie, and A. Navarro, Vegetable Raw Materials Characterization: Chemical Composition Analysis and Comparison Between Materials, pp. 347–362, 2025, doi: [10.1007/978-3-031-92874-1_28](https://doi.org/10.1007/978-3-031-92874-1_28).
- [34] ASTM D559017 (2021), Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay, Nov. 01, 2021, ASTM International, West Conshohocken, PA, USA, doi: [10.1520/D5590-17R21](https://doi.org/10.1520/D5590-17R21).