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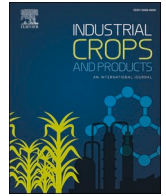
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Assessment of fungal development risk on bio-based thermal insulation

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ABSTRACT

Integrating bio-resources into building materials appears as a key point in reducing the environmental impact of the construction industry. Hemp fibre, a locally sourced and annually renewable plant resource, is particularly well-suited for insulation applications. This article aims to determine whether the environmental conditions to which loose-fill thermal insulation in an attic is exposed promote fungal development. To investigate this, the insulation solution is applied in the attic of a demonstration house, and its in-situ behaviour is continuously monitored. Sensors are embedded within the insulation layer to measure temperature and relative humidity at various depths over an 18-month period, capturing the extreme conditions the material encounters. Such conditions are then considered to investigate, in laboratory, the risk of fungal development on hemp fibre. After inoculation and incubation, fungal growth is evaluated through visual analysis and colony-forming unit counts. An isopleth of the tested hemp fibre is then constructed, and the relationship between fungal development and available water is explored through the sorption curve. The results indicate that the risk of fungal growth is reduced under the conditions studied.

1. Introduction

The use of annually renewable plant resources that do not compete with food crops is a relevant solution to reduce the depletion of natural resources and fight against global warming. Indeed, these products help to reduce the grey energy associated with the construction sector and the energy consumption of buildings, both in summer and in winter. This strategy is in line with the French government directives (Re2020, France 2030).

Hemp cultivation offers numerous ecological, agronomic and economic advantages. Its roots allow the natural fertiliser element to be fixed in the soil and 1 hectare of hemp crop absorbs as much CO₂ as 1 hectare of forest (9–15 tons), providing a resource with an excellent carbon footprint. The hemp crop does not require pesticides or irrigation and improves the yield for the next crop (European Commission, 2022; FRD and Pole IAR, 2020; Interchanvre, 2020). To obtain the fibres, the hemp straw is first retted in the field to facilitate its separation. The fibres are then separated from the shiv using a dry mechanical refining process. Hemp fibres have high intrinsic technical performance. In the building sector, hemp fibres and shiv are used to produce good fibrous thermal insulation and plant-based concretes with high hygroscopic, thermal and acoustic properties (Cérézo, 2005; Collet, 2004; Magniont,

2010; Glé, 2013; Amziane and Collet, 2017; Vincelas, 2019).

The organic composition of bio-based insulation materials makes them susceptible to fungal growth. Cellulose is easily metabolised by micro-organisms (Le Goué, 2019; Méheust, 2012; Reboux et al., 2010). Fungal growth on a material depends on several factors: the material's composition, water content, pH, physical and chemical properties, the nature of the fungi (enzymes that promote the biodegradation of the substrate) and environmental conditions (temperature (T), relative humidity (RH)) (Zine Filali, 2023).

Various methods exist for assessing the risk of fungal growth. In a comprehensive review, (Lai et al., 2024) detail and compare experimental approaches for testing mould growth on building materials, as outlined in standards and literature. Key procedural steps, including sample and contaminant preparation, inoculation, incubation, and evaluation, are thoroughly analysed. Most standards recommend inoculating samples with a mixture of fungal strains using a spray of spore suspension. However, wet inoculation can sometimes hinder the germination process. To mitigate this, some studies (e.g., Tobon et al., 2020) suggest using dry inoculation methods. *Aspergillus Niger* and *Penicillium* are the more commonly inoculated strains in standards and literature. Incubation is typically conducted under conditions of high RH and T, optimal for fungal growth (24–32.5 °C), with RH and T settings

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that can be either static or dynamic. These conditions are often maintained using climate chambers or saturated salt solutions (Adan, 1994; Laborel-Préneron et al., 2018; Lax et al., 2019; Ryparová et al., 2022). The incubation period usually lasts about four weeks for bio-based materials, but for more resistant materials, it may extend up to three months, as suggested in various studies. At the end of the incubation period, fungal development is typically assessed either visually or by analysing the number and types of fungal colonies. By examining the results obtained under various T and RH conditions, it becomes possible to identify ranges of conditions that either prevent or promote fungal growth and to determine the time required for its initiation. This leads to the construction of isopleth, which defines germination times across different RH and T conditions.

Each fungal species has its own optimal T and RH for growth (Krus et al., 2001). The material-specific isopleth helps evaluate a material's resistance to mould, indicating the combinations of T and RH that either promote or inhibit fungal development. For example, (Sedlbauer et al., 2011) investigated the resistance of hemp mat insulation and wheat straw insulation to fungal growth by inoculating samples with a mixture of ten fungal species. Fungal development was monitored over a period of 100 days under constant T and RH, as the authors deemed this duration to represent the maximum delay for germination. The growth condition of the fungi was assessed through photographs. The limit isopleth, which encompasses all tested species, was subsequently identified. The results demonstrated that hemp fibre mats exhibit significantly better fungal resistance compared to wheat straw.

However, it is noteworthy that *Aspergillus niger* was not included in the inoculation, despite this species showing a strong affinity for hemp and being naturally present in the material (Deborde et al., 2022a). The isopleth for mould spore germination of *Aspergillus restrictus* (Krus et al., 2001) can serve as a reference point for comparison in this study.

The work presented is part of the INNOFIB project, led by CAVAC Biomatériaux in partnership with the University of Rennes (GCGM laboratory). Since 2009, CAVAC has been valorising locally cultivated hemp. Hemp fibres are used to produce a range of insulating materials under the Biofib' Isolation brand, while hemp shiv is used to produce hemp concrete. The primary goal of the INNOFIB project is to enhance the performance of hemp fibre-based insulation from technical, energy, environmental, health, and economic perspectives.

The project explores and validates new technologies for hemp fibre preparation and treatments through industrial prototyping. The innovative processes developed significantly improve the thermal conductivity of hemp fibres while also enhancing their fungal resistance and fire reaction. The resulting loose-fill insulation is applied and tested under real-world conditions in a demonstrator, specifically the attic of an occupied house. A monitoring system is set up to characterise its in-situ behaviour, with sensors placed within the insulation layer. Over an 18-month period, T and RH are measured at various depths.

The aim of this article is to assess whether the environmental conditions created within the loose-fill thermal insulation in an attic promote fungal growth. The demonstrator is in the mild temperate, fully humid, warm summer climate of Vendée, France (Beck et al., 2018). The environmental conditions observed on site are then considered in the protocol developed to investigate the risk of fungal growth on bio-based insulation materials. After dry inoculation and incubation, fungal development is evaluated both visually and through colony-forming unit (CFU) counts. Laboratory tests simulate extreme in-situ conditions, and an isopleth of the tested hemp fibre is constructed to assess the potential risk of fungal growth.

2. Materials and methods

2.1. Hemp fibres and INNOFIB product

Hemp fibres are produced in Vendée by the agricultural cooperative CAVAC. The hemp variety used is *Futura 75* (*Cannabis sativa* L.). The

fibres are obtained by grinding the hemp straw through several dry mechanical refining processes. The risk of fungal development on untreated hemp fibres was evaluated in laboratory. When manually applied, the bulk density of the fibres was measured at $Mv_{\text{fibre}} = 63.7 \pm 1.8 \text{ kg/m}^3$.

The INNOFIB product used in the demonstrator, is a mix of refined hemp and recycled cotton fibres. The exact composition is confidential. Some geometrical characteristics of the fibres have been investigated (Colson et al., 2023), Fig. 1 and Table 1 summarise the results. The product was packaged in 10 kg bags. It was blown in the attic with an *ISOBLOW Mini Plus - ISOCELL* carding machine achieving a bulk density of 17.2 kg/m^3 . The dry thermal conductivity, measured by the flux metric method (AFNOR, 2001; CSTB and LNE, 2018a, 2018b), was $51.23 \text{ mW}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ at a density of 17.7 kg/m^3 .

2.2. Monitoring

The demonstrator is a retrofit project located in the south Vendée, France. It is a single-storey home, constructed with prefabricated concrete slabs fixed to a steel frame for the walls and a steel framework for the roof. The studied area corresponds to the attic (Fig. 2) located above two bedrooms, a bathroom and an open kitchen. The insulation is laid directly above existing BA13 plasterboard (thickness: 125 mm, thermal conductivity $0.3 \text{ mW}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$). Following French regulations (CSTB, 2020), there is no vapour barrier required, and none is present. The house is heated and cooled by a heat pump and a wood-burning stove. Four residents occupied the house during the study.

Monitoring of the site began in May 2022, under the original thermal insulation conditions (glass fibre) and the INNOFIB insulation solution was applied in October 2022. The monitoring ended in October 2023, throughout the 18-month monitoring period, heat flow, T and RH values were recorded at different locations including inside the rooms, in the attic ambient conditions and across the thickness of the attic floor. This article focuses on T and RH data, in relation to the risk of fungal growth.

T and RH are measured with Sensirion SHT35 sensors, which have a typical accuracy of $0.1 \text{ }^\circ\text{C}$ and 1.5 \%RH . These sensors are connected to a Daqbox V1-Stepat acquisition box. Prior to in-situ implementation, the sensors were tested with salt solutions and a calibration law was identified. In situ, sensors are fixed on wooden scales, to place the sensors at different heights within the insulating layer, ensuring no overlap (Fig. 3 (a-b)). There are also sensors taped at each interface. The environmental sensors are protected by an aluminium tube to avoid radiative exchanges and placed at a distance of more than 10 cm from the surfaces (Fig. 3(c)).

The SHT35 sensors are synchronised to record a measurement every 3 min. Weather data, including temperature, humidity, rainfall, cloud cover, and wind speed, were obtained from the *MeteoBlue* database each month. The *Meteoblue* station is located 570 m from the demonstrator. Monthly comparisons between the outdoor sensor readings and *MeteoBlue* data confirmed the consistency of the collected data.

The data processing and analysis allows to:



Fig. 1. Laboratory hemp fibre.

Table 1
Diameter and length range distribution.

Materials	Diameter (μm)		Distribution (%) by length range (mm)				
	Av	sd	> 150	150–100	100–50	50–20	< 20
INNOFIB refined hemp fibre	69	103	0,0	0,0	1,8	9,8	88,5
INNOFIB recycled cotton fibre	16	7	0,0	0,0	0,0	0,1	99,9
Laboratory hemp fibre	101	100	0	0	0	0	100

- calculate the vapour pressure (Pv) at each measurement point from the T and RH values,
- plot the kinetics of T, RH, Pv at each location,
- plot the T, RH and Pv profiles at different times over a selected time range.

2.3. Microbiological analysis

In order to evaluate the fungal resistance of hemp fibres, it was necessary to develop a reliable protocol that would ensure the fungal development on hemp fibres and also provide a quantitative result. To this purpose, development was initiated (Deborde et al., 2022a) following previous approaches (Tobon Monroy, 2020). The dry inoculation used for the lab tests simulates the case of natural spread of spore

carried by the wind in the attic.

2.3.1. Inoculation

The fungal development is assessed using 3 strains:

- 2 strains of *Penicillium sp.* isolated from bio-based materials exposed to high humidity
- 1 strain of *Aspergillus niger*, corresponding to that used by (Tobon Monroy, 2020).

The selected fungal strains are initially cultivated in flasks (Fig. 4(a)) on Dicloran Rose-Bengal Chloramphenicol (DRBC, Biokan Diagnostics) culture medium at 25 ± 2 °C until sporulation which typically take 3–5 days of incubation. This culture medium is known for its selective ability to grow moulds and inhibit bacterial growth thanks to the inclusion of chloramphenicol, an antibiotic. Additionally, the medium supports dense mould growth, facilitating easier quantification of the developed colonies.

Fungal inoculation is performed using the dry inoculation method developed by (Tobon Monroy, 2020). Samples and empty Petri dishes are randomly placed in a sealed box as shown in Fig. 4(b-c). Dry air ($D = 10 \text{ l}\cdot\text{min}^{-1}$) is directed through a network of tubes from the spore-containing vials into the box. Air circulation is maintained for 15–20 min per vial. The inoculation of the three strains is carried out sequentially. Following inoculation, a rest period of 1 h is allowed to enable the spores to settle onto the samples.

To quantify the spores, those deposited on 5.5 cm diameter Petri dishes are recovered using an extraction solution and then counted using



Fig. 2. (a) External and (b) internal views of the studied attic.



Fig. 3. (a) Sensor position, (b) wooden scale supporting the T and RH sensors, (c) sensor inside the room.

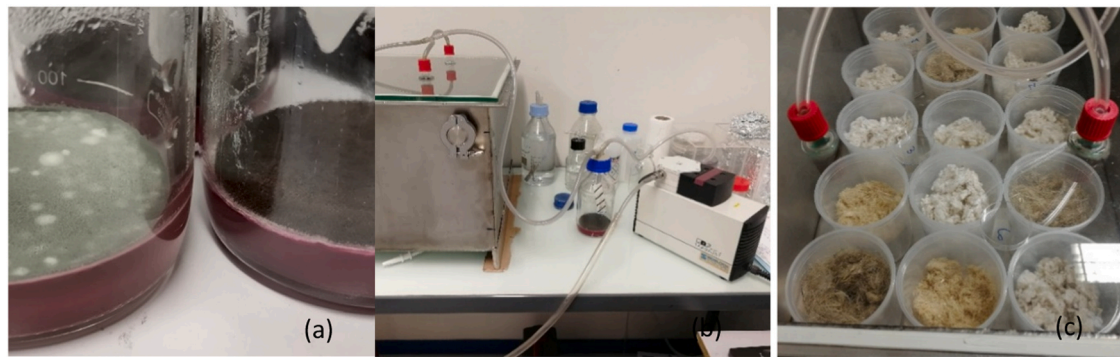


Fig. 4. (a) Culture flask for inoculation, (b) sealed box connected to the pump, (c) sample during inoculation.

the Colony Forming Unit (CFU) method. This process validates the number of spores inoculated.

2.3.2. Incubation

The specific T and RH conditions during incubation are selected regarding the result of the demonstrator, with T ranging from 10 to 23 °C and RH ranging from 70 % to 90 %. The T is controlled using a climate chamber while RH is managed with saturated salt solutions (Table 2). The equilibrium RH of the selected salts is a function of T as defined in (Greenspan, 1977).

The device consists of a double-box system: a small perforated plastic box containing the sample is suspended in a larger box. The saturated salt solution is placed in the bottom of the larger box. A sealed lid ensures that the system remains airtight. This setup is placed in a climate chamber (Fig. 5(a)). Some salts are highly sensitive to impurities, which can alter their equilibrium RH. In this setup, hemp dust from the sample can occasionally fall into the solution, potentially affecting the results. To address this, one of the three samples is instrumented for each condition. The T and RH conditions inside the boxes are monitored using SHT35 sensors inserted tightly through the lid of the assembly (Fig. 5(a)).

During the 28 days of incubation, the boxes are kept closed and observed from Monday to Friday. The first day of visible fungal development is quoted. The development may appear as filaments emerging from underneath the perforated plastic box (Fig. 5(b)) or on the side of the box (Fig. 5(c)). At the end, the boxes with no observed development are opened to inspect the core of the sample for any hidden fungal growth. In some case, incubation is extended until 35 days.

Although the hemp fibres used for microbiological analysis differ slightly from those in the demonstrator in terms of density and morphology, this discrepancy is not expected to affect the spores' affinity for the hemp fibres. Consequently, this should not alter the time required for visible fungal development to occur.

2.3.3. Water content

The amount of water absorbed by the fibre is also calculated from the sample mass after incubation and after drying.

The mass moisture content is calculated with Eq. (1).

$$\text{Moisture content} = w = (m_m - m_d)/m_d \quad (1)$$

Table 2
Theoretical tested conditions.

Temperature	10 °C	15 °C	20 °C	25 °C
Potassium bromide	83.75	82.62	79.23	78.57
Potassium chloride	86.77	85.92	85.11	84.34
Strontium Nitrate	90.55	88.72	86.89	85.06
Potassium Iodide	72.11	70.98	69.90	68.86
Ammonium Chloride	80.55	79.89	79.23	78.57
Strontium Chloride	75.66	74.13	72.52	70.85

With m_m for the moist mass, and m_d the dry mass.

The relative moisture content is calculated with Eq. (2).

$$\text{Relative moisture content} = w / w_{\text{sat}} \quad (2)$$

With w_{sat} the maximum moisture content calculated with:

$$w_{\text{sat}} = (1 - Mv_{\text{fibre}} / Mvr_{\text{hemp}}) \cdot Mv_{\text{water}} / Mv_{\text{fibre}} = 15.13 \text{ kg/kg} \quad (3)$$

With Mvr_{hemp} the reel density of hemp fibre = 1800 kg/m³ and Mv_{water} = 1000 kg/m³.

2.3.4. Colony forming unit (CFU) count

To quantify fungal growth, a biological count of the number of spores per unit area is made. The method used is that proposed by (Tobon et al., 2020) in Fig. 6. Few grams of the sample are taken and shaken with an isotonic extraction solution composed of 10⁻² M MgSO₄ and 0.25 % Tween 20. This solution is then serially diluted in 1:10 increments. From each dilution, 0.1 ml is spread onto DRBC (Dichloran Rose Bengale Chloramphenicol) culture medium. The fungal concentration, measured in CFU per cm², is determined after incubating the inoculated plates at 25 ± 2 °C for 3 days. The CFU count per cm² is compared with the number of spores inoculated onto the sample. If the post incubation CFU count is one log higher than the initial spore count, it indicates fungal growth. Conversely, if the count is equivalent to the initial spore count, the spores may have survived without growing. A decrease of more than one log in CFU count suggests that the spores have decayed.

3. Results

3.1. Building monitoring

The data recorded from winter 2022 to summer 2023 in the attic floor, insulated with the INNOFIB solution, are selected for analysis. The evolution of Pv is calculated from T and RH measurements. The periodic signal obtained leads to the calculation of the phase shift and the signal damping between indoor and attic conditions.

3.1.1. Winter conditions

Fig. 7 illustrates conditions during the coldest recorded week in February 2023. During this period, indoor temperature ranged from 15.0 to 28.8 °C while attic temperature varied from -8.5 to 17.4 °C. The indoor RH fluctuated between 28.3 % and 55.6 % whereas the attic RH went from 29.3 % to 91.6 %. The Pv is therefore always higher in the room than in the attic, inducing a moisture flux primarily moving from the indoor environment to the attic.

The thermal response of the ceiling is influenced daily by both the indoor and outdoor conditions. Outdoor temperature changes are mitigated by the effects of rapid indoor variations, such as those caused by the stove and forced-air heating (ambiance room curve in Fig. 7). These temperature changes propagate through the thickness of the insulation

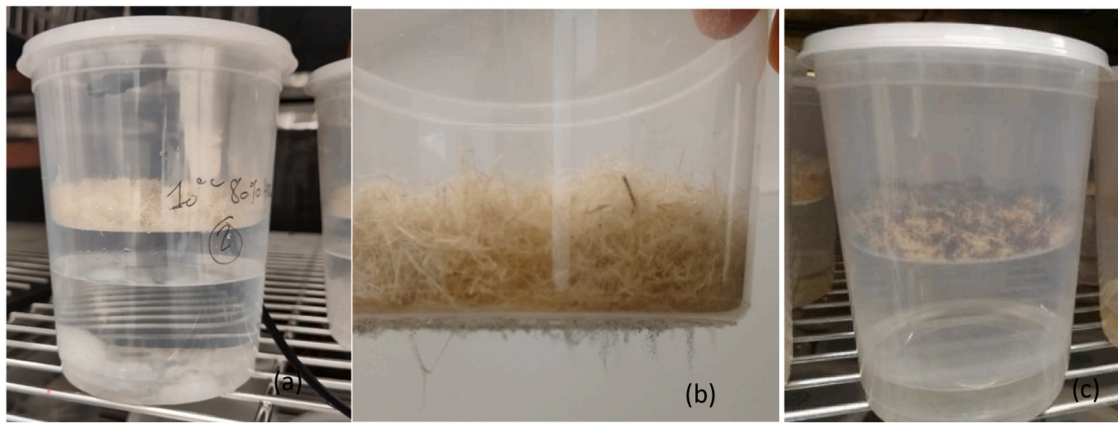


Fig. 5. (a) Sample and sensor during incubation, (b) and (c) fungal development.



Fig. 6. Biological counting method, translated from (Tobon Monroy, 2020).

layer with associated damping and phase shift, affecting both sides up to a depth of 23.6 cm. A more detailed analysis of phase shifts and damping effects between the indoor and the attic conditions will be conducted during periods without heating (e.g., spring and summer).

The hygric response within the insulation is significantly damped, with minimal daily RH variation (amplitude of RH about 60 % outside and 8 % at 6.5 cm from the plasterboard under the insulation). Major RH fluctuations occur over weekly or seasonal periods.

December 2022 was the wettest month on record (Fig. 7). Rainy days were those with warmer T and higher RH level, creating favourable conditions for fungal development during the winter period. Indeed, between the 22 December 2022 and the 31 December 2022, at 30.4 cm from the plasterboard, the average RH was 82.5 % with a standard deviation of 1.9 and the average T was 14.40 °C with a standard deviation of 1.9 °C.

3.1.2. Summer conditions

During the summer period, Fig. 8 illustrates the hottest recorded week of the August 2023 heatwave.

The hygrothermal behaviour of the insulation layer is analysed by comparing the T, RH and Pv values at the insulation - plasterboard interface with those in the attic. The indoor side of the ceiling is

significantly influenced by internal heat sources and occupant behaviour, such as window openings, which contribute to heating the house.

On the 21 August 2023, the T-phase shift between the attic environment and the insulation - plasterboard interface was 6 h and 46 min (51.8 °C at 14 h 51 in the attic, 29.2 °C at 21 h 15 under the insulation). On the 22 August 2023 the phase shift was 5 h and 27 min (52.4 °C at 15 h 15 in the attic, 29.4 °C at 20 h 42 under the insulation).

On the 21 August 2023 the damping factor was 0.10 with a temperature variation of 3.1 °C at the insulation-plasterboard interface (29.2 °C and 26.1 °C) and a variation of 32.5 °C in the attic (51.8–19.2 °C). On the 22 August 2023, the damping factor was 0.08 with a variation of 2.6 °C at the insulation-plasterboard interface (29.4 °C and 26.8 °C) and a variation of 32.7 °C in the attic (52.4–19.6 °C).

During the summer, at the peak of temperature evolution, the highest Pv is observed at a depth of 30.4 cm within the insulation layer, indicating ongoing desorption. The RH variation exhibits a greater phase shift compared to temperature (Fig. 9). On the 21 August 2023, the RH phase shift between the attic environment and the insulation - plasterboard interface was 12 h and 45 min (maximum 81.4 %RH at 7 h 24 in the attic and 65.1 %RH at 20 h 08 under the insulation). On the 22 August 2023, there was 12 h dephasing (77.6 %RH at 06 h 57 in the attic, 64.4 % RH at 18 h 57 under insulation).

The highest humidity levels within the insulation layer, under warm temperature conditions, occur during rainy days. The Fig. 10 illustrates this scenario. The RH inside the insulation remains stable. Between the 1 August 2023 and 7 August 2023, at a depth of 6.3 cm from the plasterboard the average RH was 65.1 % with a standard error of 1.6 and the average T was 24.4 °C with a standard error of 0.5 °C.

3.1.3. Conditions reached

Regarding the T and RH variations during winter and summer, it is observed that the T fluctuates over a 24-h period while the RH changes over approximately 7 days. Therefore, Fig. 11 presents the calculated one-week average values, measured at each sensor position. This figure highlights the extreme conditions reached within the thermal insulation layer. For fungal development considerations, it will be important to check that mould growth is not initiated over 7 days at such high RH and T levels.

3.2. Isoleth

3.2.1. In situ data with literature isopleth

The data presented in Fig. 11 are compared with the isopleth of (Sedlbauer et al., 2011), and (Krus et al., 2001) in the Fig. 12.

Referring to (Sedlbauer et al., 2011) work on hemp, the yellow zone represents conditions where fungal growth cannot be completely

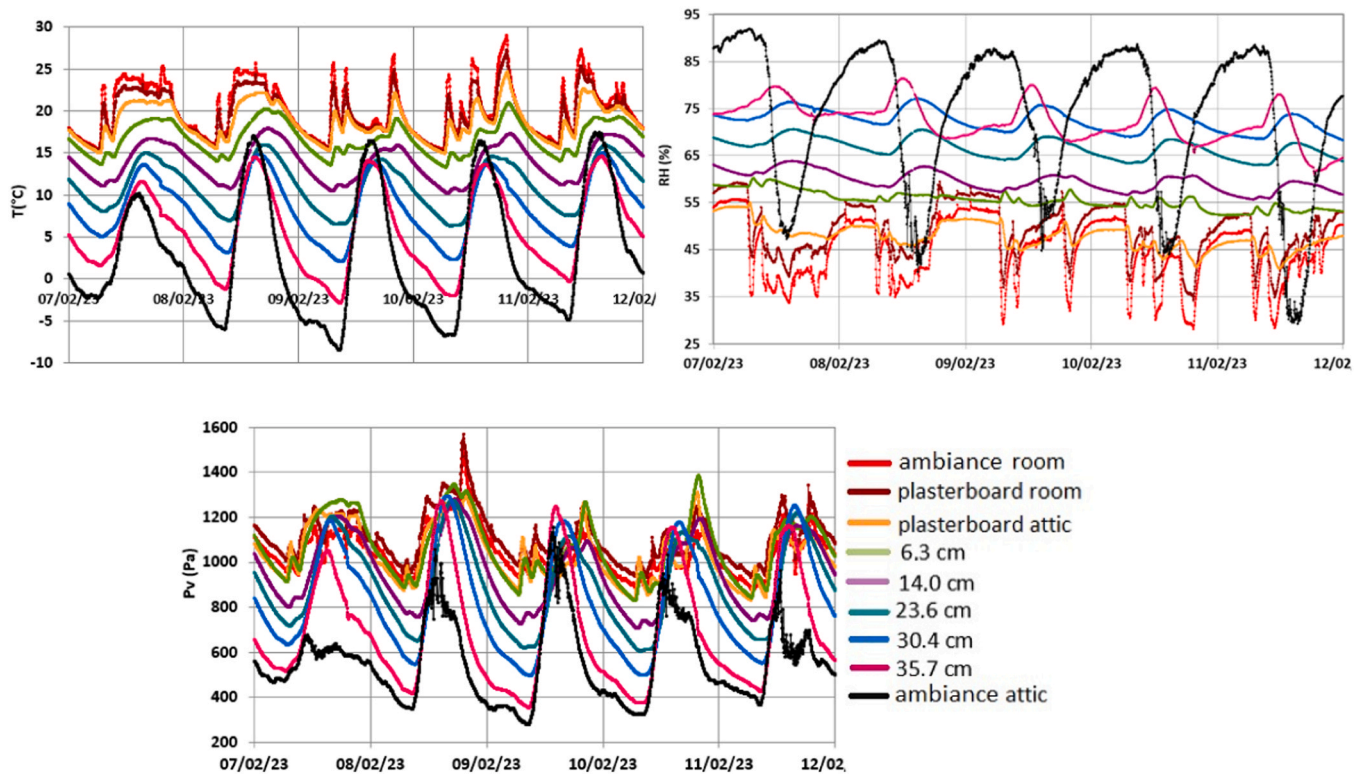


Fig. 7. T, RH and Pv variation during the coldest week of winter 2022–2023.

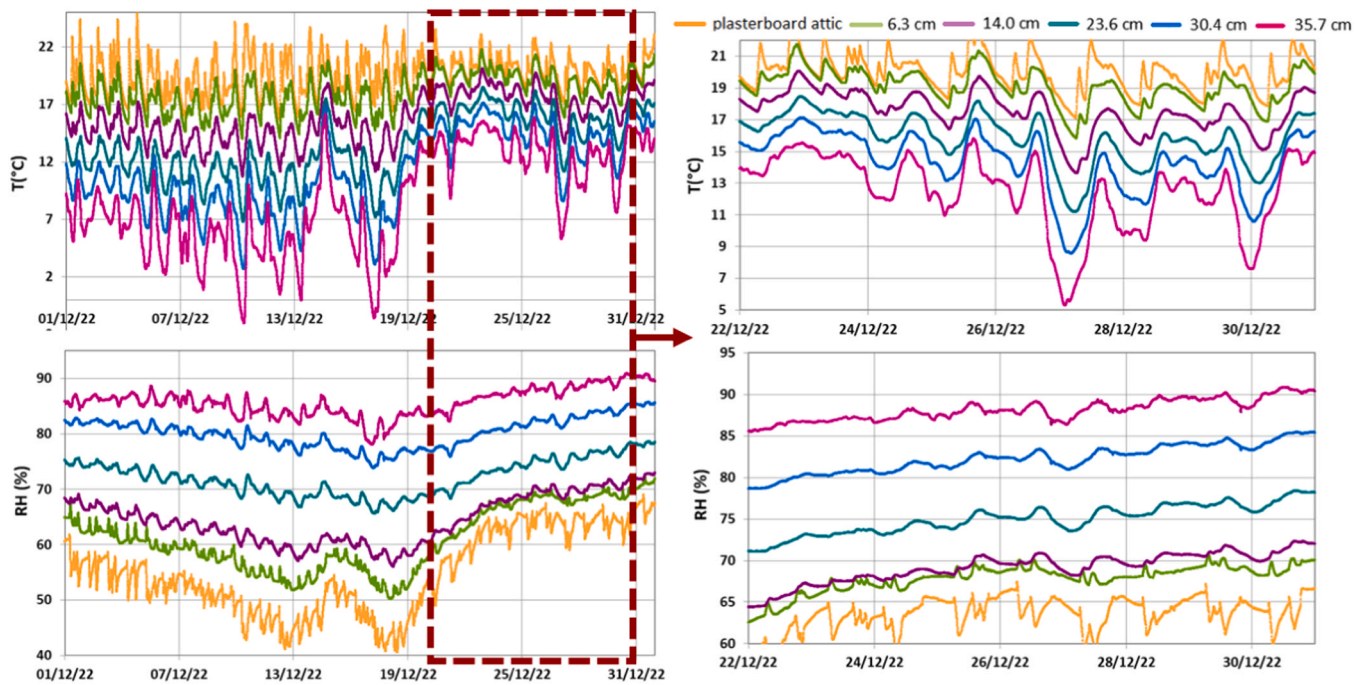


Fig. 8. T and RH inside insulation in December 2022 (distances from the attic side of the plasterboard).

excluded during the 100-days incubation period, while the green zone indicates conditions where no growth is expected. Notably, the data from the demonstrator never enter the yellow zone. Furthermore, a 100-days incubation period seems inappropriate given to the T and RH fluctuation periods evaluated using the demonstrator data.

In relation to the isopleth for *Aspergillus Restrictus* on hemp (Krus et al., 2001), some of the T and RH measurements match with curves

corresponding to spore germination after 8–64 days of incubation.

Therefore, it seems appropriate to compare the demonstrator measurements to the isopleth for *Aspergillus Niger* and *Penicillium* on hemp. An incubation period of 28–35 days appears sufficient for assessing fungal risk in this context.

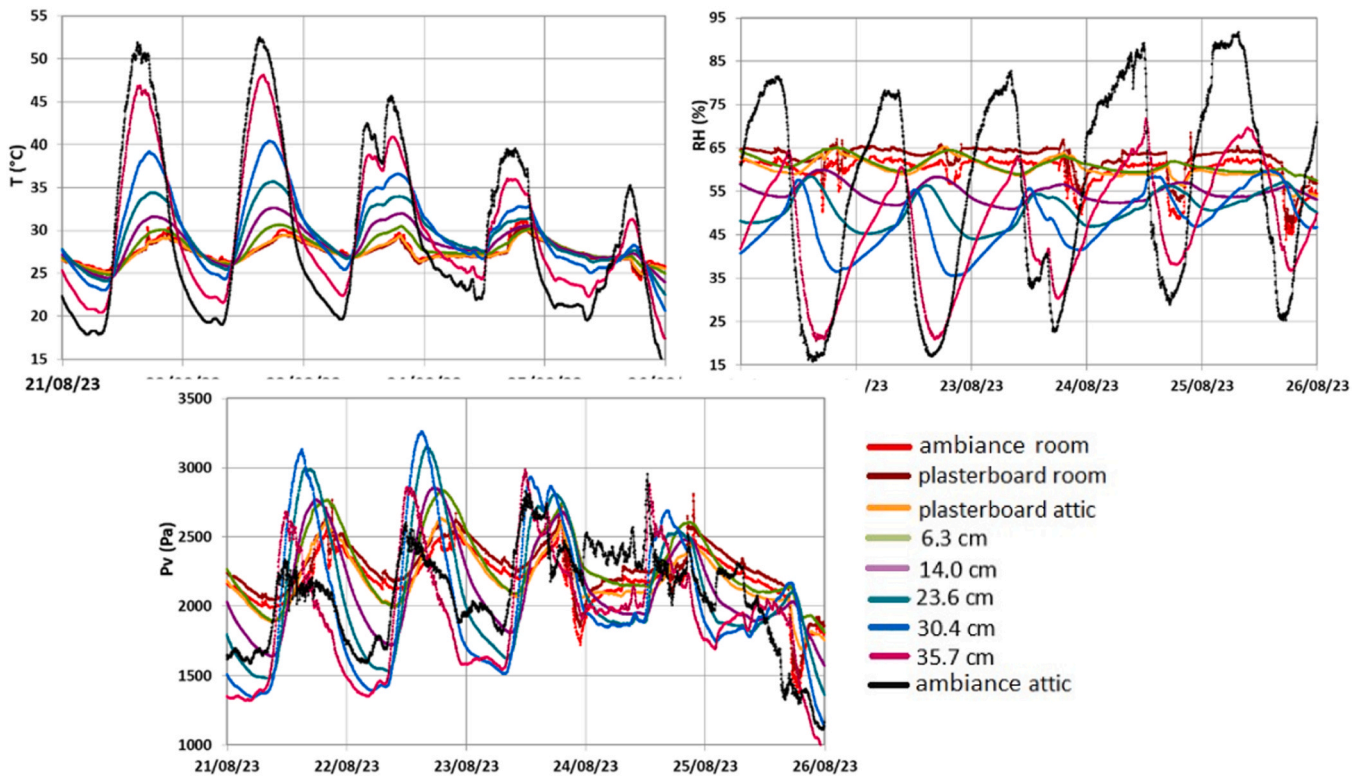


Fig. 9. T and Pv variation during the hottest week of summer 2023.

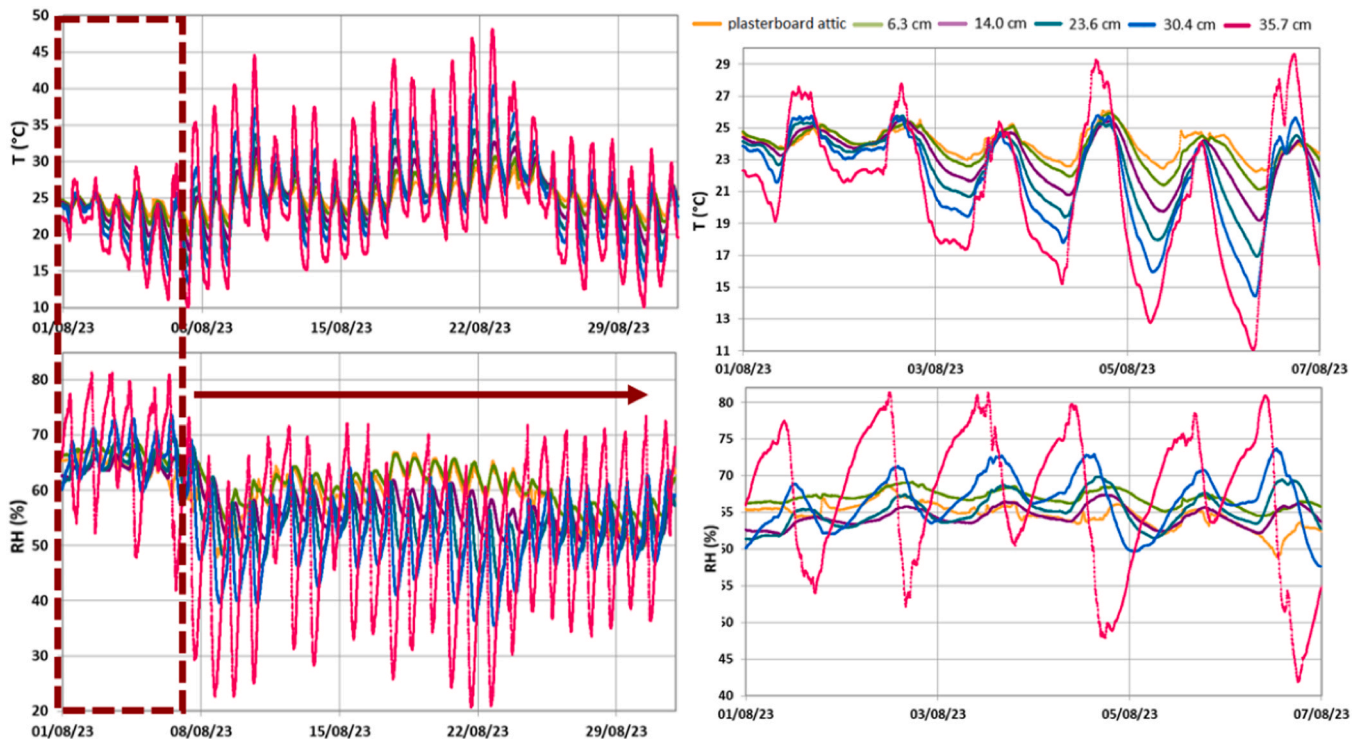


Fig. 10. T and RH inside insulation in August 2023.

3.2.2. Hemp fibre isopleth

Inoculated hemp fibre exposed to 100 % RH and 25 °C shows fungal development after approximately 7 days (Deborde et al., 2022b). The conditions for testing were selected by analysing Fig. 11 focusing on extremes such as the highest RH with the high corresponding T (10 °C –

95 % RH) and the highest T with high corresponding RH (23 °C – 70 % RH).

Fig. 13 (a) shows the number of days needed for fungal development (if there is fungal development) under various incubation conditions. The blue line corresponds to the limit conditions without fungal growth.

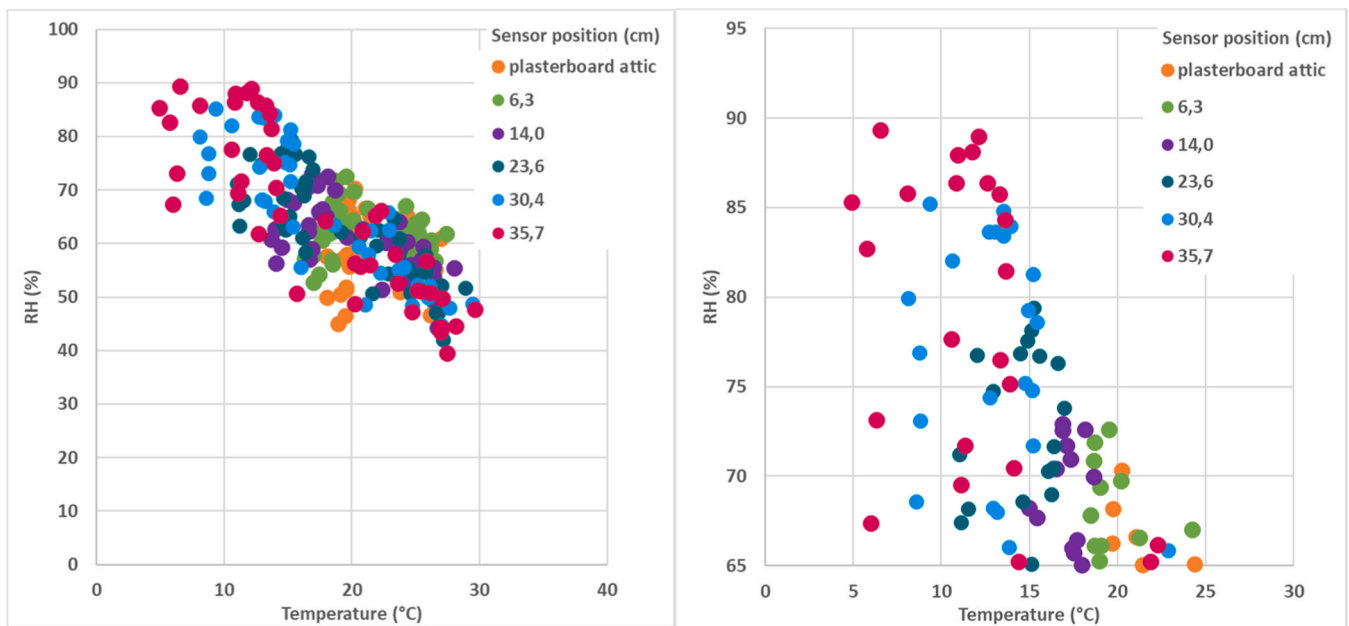


Fig. 11. T and RH measured inside insulation.

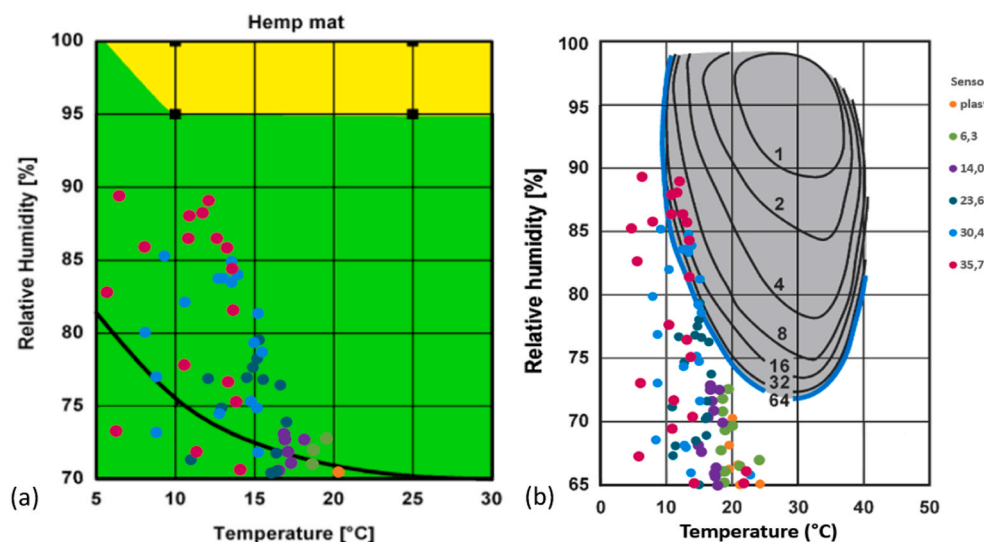


Fig. 12. In situ data with isopleth adapted from (a) (Sedlbauer et al., 2011) and (b) (Krus et al., 2001).

The results are combined with data from Fig. 11 in Fig. 13 (b).

The obtained results present some similarities with those of (Krus et al., 2001) (Fig. 12(b)). At 10 °C, fungal growth requires a minimum of 96.4 %RH and a duration of 33 days. However, even at the top of the insulation layer, the maximum RH measured over 7 days was 89.3 %RH at 6.6 °C. At 18 °C, fungal growth requires a minimum RH of 89.0 % and 22 days. The closest measurement recorded was 18.14 °C and 72.6 % RH at a depth of 14 cm from the plasterboard for one week.

For the first sampling campaign, if no fungal development was observed, the visual assessment was confirmed by spore counting (Table 3). After 28 days, the average CFU/g was one log lower than at the time of inoculation, confirming the absence of fungal growth. Consequently, spore counting was not performed for the other samples.

The isopleth for untreated hemp fibre covering temperatures from 10 to 23 °C and RH level from 70 % to 96 % for a mixture of fungal strains (*Aspergillus Niger* and *Penicillium*) indicates that the conditions required for fungal development, whether due to natural inoculation or initial

contamination, are not met in the attic. This finding is valuable under normal use conditions, without leakage, without a vapour barrier and within the Vendée climate.

3.2.3. Water content regarding fungal development

The time required for fungal development can be analysed as a function of the water content available in the vapour. Values of water content (w_v) can be calculated from the mass of vapour contained in the porosity associated with 1 kg of fibre:

$$W_v = (RHP_{Sat} / R_v T) \cdot (1 - Mv_{fibre}/Mvr_{hemp}) \cdot 1 / Mv_{fibre} \quad (4)$$

With $R_v = 461.5 \text{ J}/(\text{kg}\cdot\text{K})$, P_{sat} the vapour pressure at saturation, function of the temperature (AFNOR, 2016).

Results are presented on Fig. 14. The values of w_v remain low (0.1–0.3 g/kg). The parameter w_v appears not sufficient to simply forecast the incubation period duration required for fungal

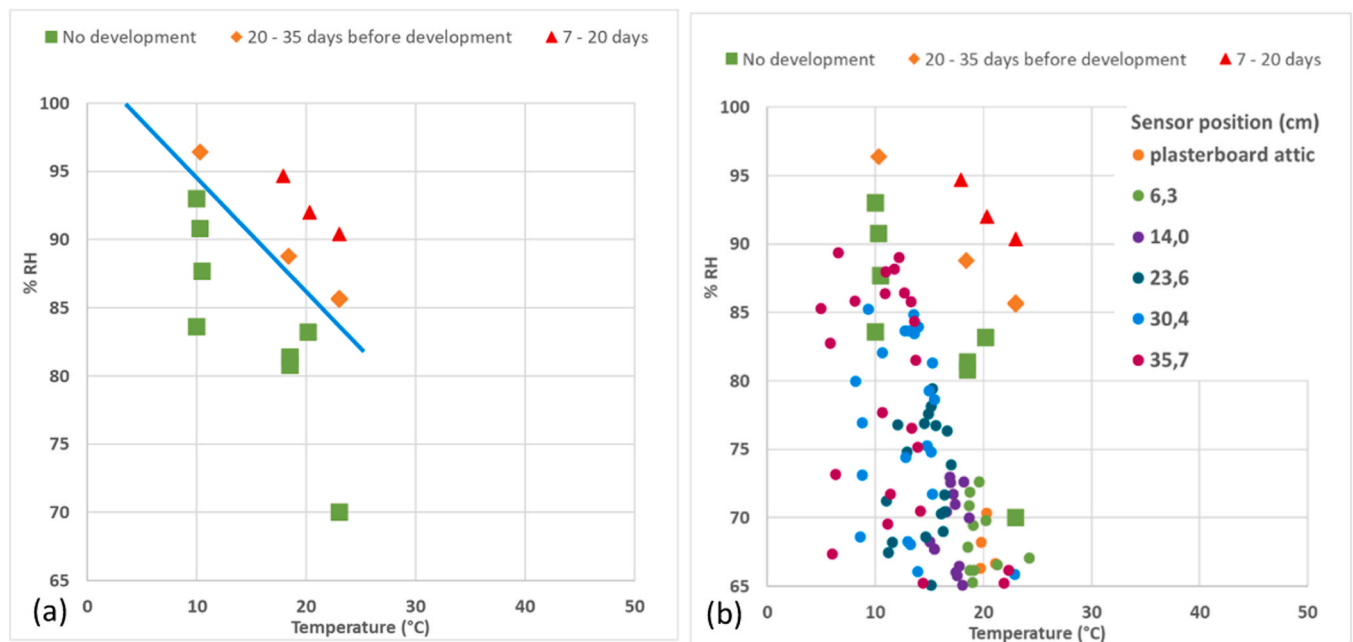


Fig. 13. (a) Hemp fibre isopleth, (b) overlay of hemp isopleth and demonstrator conditions.

Table 3

Result of spore counting.

Incubation condition	UFC/g average at 28 days	UFC/g standard error at 28 days
10 °C 80 % RH	7.3E+04	2.9E+04
10 °C 85 % RH	9.7E+04	2.4E+04
18 °C 70 % RH	1.7E+04	1.3E+04
18 °C 80 % RH	8.1E+04	6.6E+04

Inoculation 2.0E+5 UFC/g.

chosen RH. The time required for fungal development leads to the identification of zones that are helpful in identifying the minimum moisture content required for fungal development as a function of temperature.

The time required for the fungal development can be analysed as a coupled function of w and w_v . The results are shown in Fig. 16. Both parameters are influential.

In order to relate the risk of fungal development to water activity or the water availability (Beuchat, 1983), isopleth results are discussed in relation to isothermal sorption curves.

The adsorption and desorption isotherms at 23 °C measured on hemp wool are presented in (Collet et al., 2011). The sorption model used is based on the Guggenheim -Anderson -De Boer (GAB) model, which describes physical sorption mechanism. Like the BET model, the GAB model is physically valid only in the absence of capillary condensation, i. e., for low and medium relative humidities. However, this model does not accurately predict sorption behaviour at high RH values near saturation. The sorption model used in this paper corresponds to the modified GAB model used by (Oumeziane et al., 2016) and (Oumeziane et al., 2021):

$$\frac{w(RH)}{w_{sat}} = \frac{RH(1 - b)}{(1 - bRH)} \frac{[1 + (a - 1) b]}{[1 + (a - 1) bRH]} \quad (5)$$

Where a , b and w_{sat} can be fitted to experimental data. In our case, to fit the data obtained at different temperatures we can complete the model with the following equations (Oumeziane et al., 2021):

$$w_{sat} = w_{sat,0} e^{Q_{sat}/RT}$$

$$a = a_0 e^{(E_1 - E_m)/RT}$$

$$b = b_0 e^{(M_L L_v - E_m)/RT} \quad (6)$$

With $M_L = 0.018 \text{ Kg.mol}^{-1}$; $L_{vt} = 2.45 \text{ MJ.Kg}^{-1}$; $R = 8.314 \text{ J.mol}^{-1} \text{ K}^{-1}$; T temperature (K). Q_{sat} , E_1 and E_m remain to be evaluated from data obtained at different temperatures.

In this work, we assume that w_{sat} does not vary with temperature, considering that the thermal dilatation of the material remains homothetic (no change in solid volume fraction). Given that the studied RH values are high ($RH > 65 \%$), we quote a large influence of the b

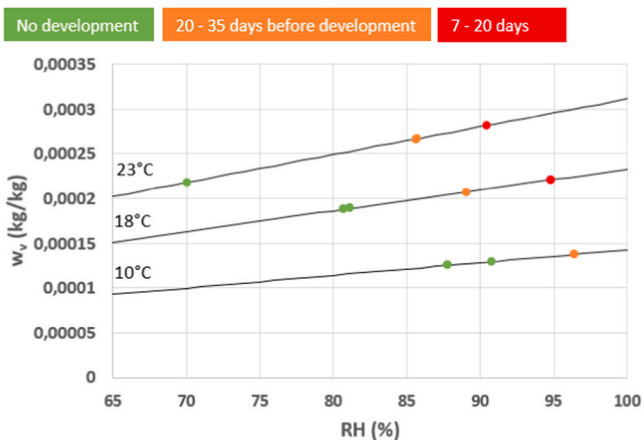


Fig. 14. Fungal development risk regarding water content (w_v) in the porosity per kg of fibre in function of RH and T.

development.

The time required for fungal development can be analysed as a function of the water content in the hemp fibre (w). After incubation, some fibres are dried to assess w . These data points are part of the adsorption curve of hemp fibre. The results of w versus incubation conditions (RH and temperature) are presented in Fig. 15. The water content w varies between 0.080 and 0.220 kg/kg. Interestingly, increasing the incubation temperature induce a lower water content at

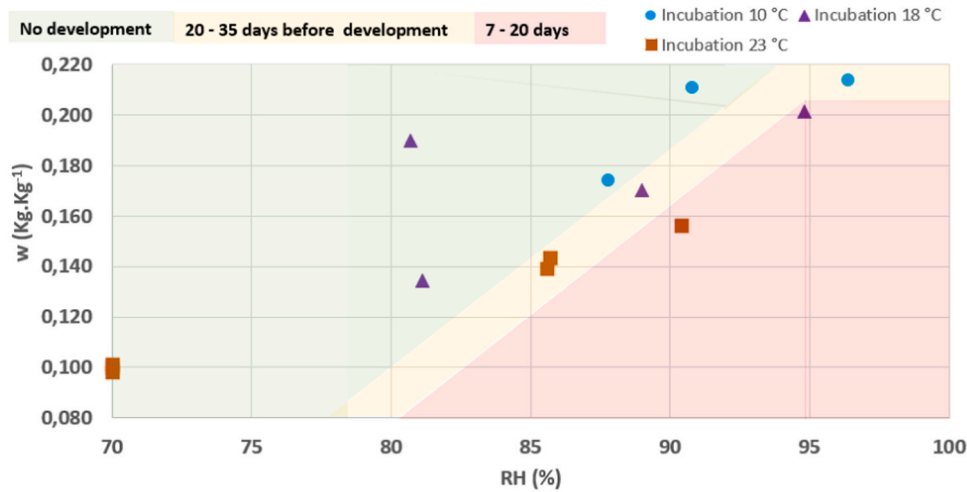


Fig. 15. Fungal development regarding RH and water content.

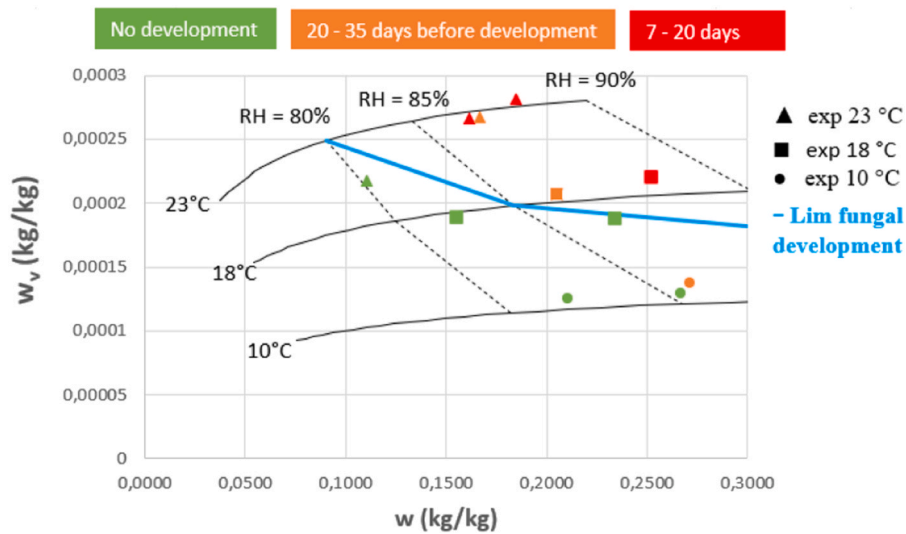


Fig. 16. Fungal development risk regarding water content in vapour (w_v) and water content in fibre (w).

parameter on the shape of the sorption curves and a small effect of the a parameter (influencing mainly the shape of the sorption curve at low RH). Consequently, the influence of the temperature on the a parameter is neglected. This assumption is supported by considering that $E_1 = E_m$: the activation energy associated with the sorption of the first layer of water molecules is assumed to be the same as the activation energy for subsequent layers. Therefore, the parameters of Eq. (6) are simplified to:

$$w_{sat} = 15,13 \text{ kg/kg}$$

a

$$b = b_0 e^{(M_L L_v - E_m)/RT}$$

The curves are fitted to the experimental data presented in Fig. 15 by fitting a, b_0 and E_m . The parameters a and b are first identified from the experimental data obtained at 23 °C, then b_0 and E_m are identified to fit with the data measured at 10 and 18 °C:

- a = 0.5
- $b_0 = 1.0396$
- $E_m = 44,200 \text{ J/Kg}$.

Fig. 16 presents the calculated values of w from the sorption model

and w_v at 23 °C, 18 °C and 10 °C (solid curves for RH ranging from 65 % to 100 %). The impact of the temperature on the moisture content of the hemp fibre is highlighted. The higher the temperature, the lower the moisture content for a given RH (dashed curves). Although the fit with the experimental data is not perfect, the graph provides useful insights into the RH value that can be used at a given temperature to avoid the fungal development, thus complementing the isopleth analysis.

Regardless of the temperature, it appears that the limit of fungal development (from Fig. 13 (a) on Fig. 16) is predominantly influenced by w_v . This indicates that fungal development is more strongly influenced by the water available in the ambient air vapour than by the water adsorbed within the material. Consequently, the role of water activity on fungal development is not as significant in this context.

4. Conclusion

This study highlights the performance of INNOFIB solution, a bio-based insulation materials with high hygroscopic properties tested during 18 months in real conditions. The demonstrator has provided valuable insights into the conditions achieved in the attic and within the insulation layer. Despite a low density (17.2 kg/m^3) and a low thermal conductivity (close to $51.23 \text{ mW}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$) the insulation led to a

thermal phase shift of about 6 h during summer and a damping of 0.09, reaching a satisfactory compromise. T and RH variations during both seasons followed distinct cycles. T fluctuated over 24 h, while RH fluctuated weekly. The one-week average T and RH values measured at different sensor locations, highlight extreme conditions encountered within the insulation. These values are used to define the protocol for verifying that mould growth is not initiated over 7 days under such conditions.

To assess the fungal resistance of hemp fibres, laboratory-scale tests were conducted replicating the conditions observed in the demonstrator. The developed method allows precise control over T and RH to which the inoculated samples are exposed. The results are consistent and reliable. Isopleths for untreated hemp fibre were constructed for the range of 10–23 °C and 70–98 % RH using a mixture of fungal strains (*Aspergillus Niger* and *Penicillium*).

The comparison between the untreated hemp fibre isopleth and the demonstrator data indicates that the conditions for fungal development are not met for the species studied. This result remains valuable only in the case of normal conditions of use, without leakage and without a vapour barrier, in the Vendée climate. It would be valuable to extend this study to other climates. However, for tropical climates, for example, other fungal species should probably be inoculated.

The time required for fungal development was further analysed as a function of the water content in the vapour surrounding the fibre and the water content adsorbed in the hemp fibre (water activity). Both parameters significantly influenced the fungal development.

The results based on the fit of a judicious sorption model, show that fungal development depends more on the water in the vapour than the adsorbed water content. This emphasises the importance of controlling the ambient air humidity content, especially under high humidity conditions.

Further research could investigate the survival of fungal spores under extreme conditions, such as at 50 °C and 30 % RH, which can occur during peak summer weeks. This could provide deeper insights into the long-term durability and fungal resistance of bio-based insulation materials under varying climatic conditions.

CRedit authorship contribution statement

Christophe Lanos: Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Data curation. **Yves Andres:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **Lily Deborde:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Florence Collet:** Writing – review & editing, Validation, Supervision, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Deborde Lily reports financial support was provided by The French Agency for Ecological Transition. If there are other authors, they 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.

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