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A Novel Biocomposite Made of Citrus Peel Waste and Mushroom Mycelium: Mechanical, Thermal, and Bio-Repellency Studies

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Abstract

The growing environmental pollution and the imminent depletion of natural resources highlight the need for alternative building materials derived from renewable sources, including those that promote waste recycling and biodegradability. One promising alternative is biocomposites produced from filamentous fungal mycelium. In Argentina, orange and lemon peels are among the most abundant organic waste generated by the citrus industry. This study explores the development of a sustainable insulating biocomposite using *Pleurotus ostreatus* mycelium grown on mixtures of citrus peels, paper, and cardboard. The test specimens were prepared using varying concentrations of these components. The resulting fungal biocomposite exhibited a density approximately ten times higher than expanded polystyrene, with drying shrinkage ranging from 28% to 51%, depending on the formulation. Key properties were evaluated, including compressive strength (σ_{10} = 7–33 kPa), bulk density (ρ = 152–181 kg/m^3), and thermal conductivity (λ = 0.29–0.36 W/mK), indicating advantageous performance for thermal insulation in construction applications. Specimens containing orange peel also demonstrated repellent activity against *Triatoma infestans*, main vector of transmission of Chagas' disease, attributed to the residual limonene content retained from the citrus peels. This fungal biocomposite aligns with principles of green chemistry and circular economy, offering a biodegradable, low-impact solution with potential use in construction. The citrus waste proved to be an effective substrate for mycelial growth, producing a material with desirable mechanical and thermal properties, and added resistance to biodeterioration.

Keywords: citrus peels; circular economy; waste reuse; insulating material; insect bio-repellency; Chagas' disease

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1. Introduction

In recent decades, various alternatives have been explored to replace plastics and reduce the environmental harm caused by their improper disposal and their long-lasting resistance to degradation after use [1]. Latin America is the third largest contributor to plastic waste in the oceans, with Peru top of the list, followed by Venezuela and Argentina, generating 0.44, 0.25, and 0.18 kg of plastic waste per capita per day, respectively [2]. The

construction sector is increasingly recognized as a key player in the transition towards a more resource-efficient future. Fungal biocomposite materials, along with other bioplastics and biobased materials that are gaining significant momentum in packaging or architectural applications, represent one promising solution to the environmental challenges [3–7]. Mycelium is a network of hyphae produced by certain fast-growing filamentous fungi. In materials developed through fungal biotechnology, organic waste (such as agricultural and urban residues) is upcycled, and the resulting biocomposite, created via solid-state fermentation, is fully biodegradable at the end of its life cycle, aligning with the principles of the circular economy. In fungal biocomposites, the mycelium that grows on the waste substrate acts as a natural binder, offering a promising alternative to plastic-based materials in the construction industry. These materials possess a lower embodied energy and, in addition, offer a sustainable solution to the depletion of fossil resources utilizing renewable inputs.

However, a common challenge faced by biocomposite building materials is their vulnerability to biodeterioration caused by animals, insects, and microbes [8]. In the case of fungal biocomposites, several mechanisms can lead to this; a notable example is through the growth of competing microorganisms during the incubation phase (Figure 1), or through insect activity, as certain species burrow into and nest within the fully dried material (Figure 2) [9].

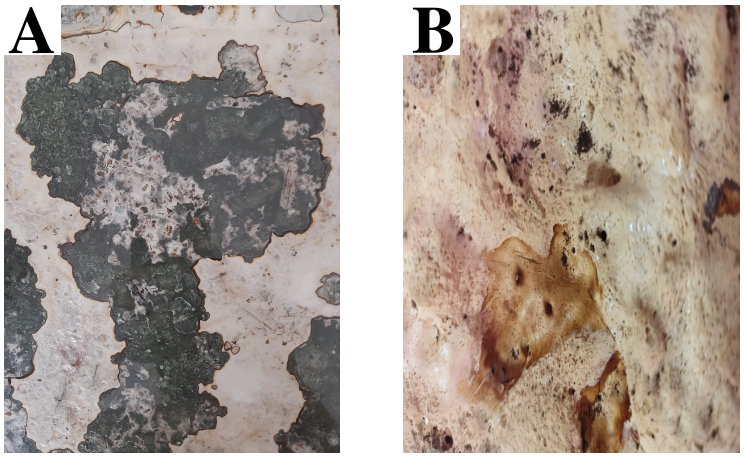


Figure 1. Biodeterioration affecting fungal biocomposites during the growth phase. (**A**) *Aspergillus* spp. (**B**) *Mould*.

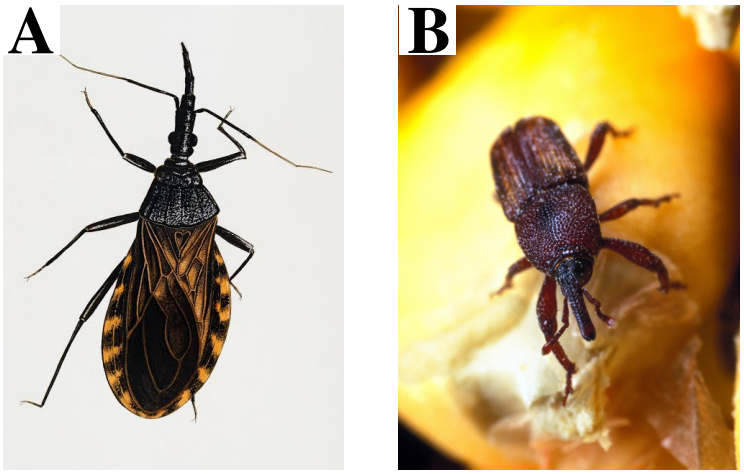


Figure 2. Physical appearance of *Triatoma infestans* (**A**) and *Sitophilus zeamais* (**B**).

Bio-repellency refers to the capacity of a material or composite to resist the adhesion and growth of living organisms, including bacteria, fungi, algae, lichens, insects and other organisms. This property helps prevent biological colonization on either the surfaces or within the matrices of composite materials. Traditionally, bio-repellency has been achieved through chemical treatments, which often leave behind toxic residues in both biotic and abiotic environments [10]. In light of the detrimental consequences associated with insecticide application, an increasingly active area of research focuses on the development of effective and environmentally safe alternatives for pest management. In contrast to extermination, a biodiversity-respecting approach prioritizes repelling disease vectors to mitigate human health risks. In architectural settings prone to microbial proliferation, bio-repellency plays a crucial role in preventing contamination and the spread of pathogens.

A variety of insects and microorganisms can affect buildings [11–13]. Figure 2A shows *Triatoma infestans*, commonly known as "vinchucas", a blood-feeding insect and the primary vector of the protozoan *Trypanosoma cruzi*, the parasite responsible for Chagas disease, also known as American trypanosomiasis [14]. More than 7 million people worldwide are estimated to be infected with *T. cruzi*, leading to more than 10,000 deaths every year [15,16]. Although a condition of increasing global presence, Chagas disease is found mainly in endemic areas of 21 continental Latin American countries (Argentina, Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Uruguay, and Venezuela), where transmission is largely related to the presence of the insect vector. Today, more than 100 million people are considered at risk of infection [17]. This insect typically nests in homes with unplastered walls, cracks, and roofs, as well as in nearby structures such as chicken coops, animal pens, and storage areas. Control of this vector has traditionally relied on the application of insecticides. However, the effects of insect repellents on *T. infestans* have been poorly studied. Identifying a potentially effective repellent could be a valuable tool for future control strategies for this public health pest [18]. For more than two decades, pyrethroid insecticides have been the cornerstone of efforts to control the proliferation of triatomine bugs in and around homes. Among these, deltamethrin has been the most extensively employed pyrethroid and has demonstrated high efficacy as a triatomicide [19]. However, in recent decades, the Argentine health authorities have reported increasing failures in the control of *Triatoma infestans*, which have been linked to varying levels of resistance to pyrethroids [20]. This resistance may be partially attributed to the rapid degradation of the active ingredients, as supported by several studies [21]. In response to these challenges, alternative strategies have been developed, focusing particularly on polymeric systems that enable the protection and controlled release of active agents. Such polymer-based formulations have shown significantly extended efficacy compared to conventional suspension concentrates, under both laboratory and field conditions [22]. Notably, recent studies have reported the potential repellent activity of the essential oil contained in *Zuccagnia punctata*, a native Argentine medicinal plant, when incorporated into poly(ϵ-caprolactone) matrix via solvent casting techniques [23]. Aromatic plants and the substances they contain have emerged as promising substitutes for traditional insecticides and repellents. Products formulated with essential oils (EOs) are considered environmentally sustainable and can offer effective personal protection against mosquitoes and other hematophagous insects [24]. In addition, EOs typically show low toxicity in mammals, degrade rapidly in natural environments, and face fewer regulatory restrictions, partly due to the long-standing cultural acceptance of aromatic plant species. Essential oils extracted from Andean plant species native to San Juan province (central–western Argentina) have demonstrated strong repellent effects against *Triatoma infestans* (Klug) (Hemiptera: Reduviidae), the main vector of Chagas disease. These oils

represent a valuable source of biodegradable, biologically active metabolites [25–27]. Previous work described the chemical profile and the insecticidal and antimicrobial properties of the essential oil obtained from *Baccharis darwinii* in Argentinian Patagonia. In that study, several major constituents with known bioactivity were identified, including limonene (47.1%), thymol (8.1%), and 4-terpineol (6.4%).

Also some aromatic medicinal species collected in Argentina, which showed significant biorepellent activity, class III *T. infestans*, stood out for having a 10% content of limonene [28]. The potential repellent activity of citrus species against other insects that affect human health, such as cockroaches and ticks, has been reported by several authors. Some additional repellent activity against the before mentioned insects could be expected from these biocomposites made from orange and lemon peels [29].

Another common pest found in building like storehouse and storage silos is *Sitophilus zeamais* (Figure 2B), a species known for causing significant damage to agricultural products in either urban and rural environments. This insect is polyphagous because it can damage several food commodities such as corn, sorghum, rice, and wheat as well as processed food products. The damage to products or stored materials has economic importance because these materials are ready for consumption and these materials have cost a lot of money for seeding, tillage, planting, maintenance, and harvesting [30].

To control pests of this kind, several strategies have been employed, including regular inspection and cleaning, proper storage practices, the use of traps, chemical insecticides, and biological control using natural predators. Nevertheless, the use of repellency as a pest management strategy remains largely unexplored, particularly through the development of materials that inherently possess repellent properties. In the construction sector, for example, chipboard has been manufactured using orange peel waste [31], highlighting the potential for integrating waste-based materials with added functional properties. However, a few reports have investigated the incorporation of EO into construction materials for insect-repellent purposes [32,33], despite existing research demonstrating the insecticidal and repellent bioactivity of essential oils [34]. Especifically, there are some previous reports showing the repellent effects of citrus peel and its constituents, such as limonene, on *Sitophilus zeamais*. A report indicates that the orange oil from *Citrus sinensis* L. against maize weevil (*S. zeamais* Motsch) has potential insecticidal characteristics, so it can be used as natural insecticides for storage pests and other hand showed lower repellency effect against maize weevil *S. zeamais* with 36% repellency [35]. A study of peel oils from *Citrus aurantiifolia* and *Citrus reticulata* cultivated in northeastern Brazil suggest that these citrus oils and the two enantiomeric forms of limonene have toxic effects on *S. zeamais* in different ways (i.e., via the cuticle, digestive system and respiratory system) as well as a behavioral effect (repellency) [36]. On the other hand, *Triatoma infestans*, there are no specific reports of repellent activity from citrus peels. However, there are reports of repellent activity from essential oils of Andean plants, which have limonene and other terpenes as their main components. *Baccharis grisebachii* essential oils collected in Argentina, stands out for the presence of limonene (47.1%), thymol (8.1%) and, 4-terpinelol (6.4%), showed promising repellent activity against *T. infestans* (average repellence 92%) [28,37].

This study aims to evaluate the bio-repellency potential of fungal biocomposites through the incorporation of citrus residues within the growth substrate. Furthermore, the mechanical and physical properties of the resulting biocomposites were analyzed, given its proposed application in the construction industry.

2. Results and Discussion

The cultivation period for the test specimens lasted 30 days under controlled temperature and humidity conditions (25 °C and 80% RH). Growth was halted once full mycelial

coverage was observed on the surface of the specimens. So, the chemical sanitation method proved effective for the treatment of citrus residues, since no contamination was observed in the fungal cultures. It is worth noting that during the immersion of citrus peels in the $Ca(OH)_2$ suspension, lime tends to precipitate at the bottom of the container, potentially resulting in uneven sanitation. One way to achieve significantly more uniform contact between the peels and the sanitizing solution is to incorporate mixing equipment into the pretreatment process, such as a concrete mixer. This stage is illustrated in Figure 3, where a whitish layer is visible, covering the sterile substrate. At the end of the incubation period and fungal growth, the dimensions and masses of the specimens were measured prior to oven drying. These measurements were repeated after drying at 105 °C until a constant mass was achieved, indicating complete moisture removal. The dried specimens of fungal biocomposite appeared whitish, were lightweight, and retained a noticeable orange scent due to the presence of essential oils. Figure 4 shows the specimens before and after the drying process.

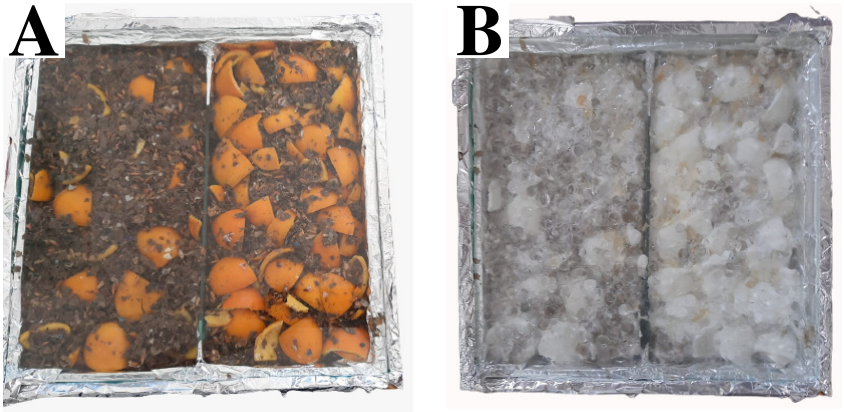


Figure 3. Incubation of **O-50** and **O-75** (**A**) day 1 and (**B**) day 26.

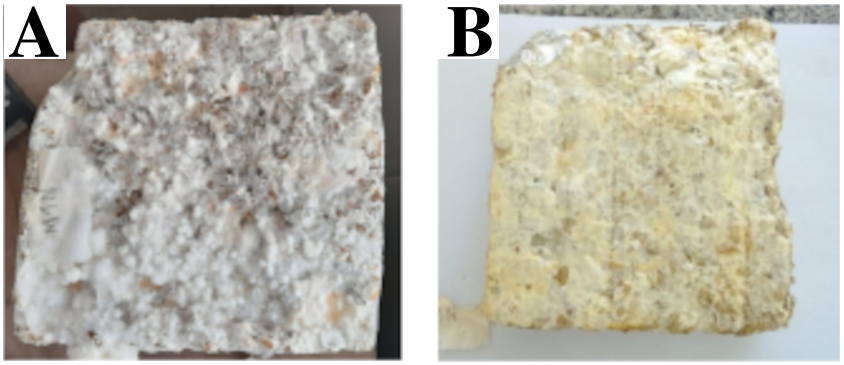


Figure 4. Sample **O-25** obtained after 30 days of incubation (**A**) before drying and (**B**) after drying.

2.1. Physical and Mechanical Properties

Table 1 presents the volumes of the specimens in wet and dry state, as well as the calculated volume variation and drying shrinkage (DS). In the orange peel series, the dry volume tends to decrease as the percentage of peel increases (Table 1, entries 1 to 5). This is reflected in the DS values, which rise with higher orange peel content. Specimens **REF** and **O-25** exhibited shrinkage values of 20% and 28%, respectively. When the peel content reached 50% *w*/*w* (**O-50**), DS increased to 36%, and in the formulations with the highest peel content, **O-75** and **O-90**, the DS reached 42% and 51%, respectively. The lower shrinkage observed in **REF** suggests it's a result of the reduced void volume in the paper-based formulation, which lacks citrus content-typically responsible for creating larger voids within the structure. It was also observed that the mycelium colonized the

surface of the specimens, forming a coating-like outer layer and acting as a binder within the core. Another notable feature was that the **REF**, **O-25**, and **O-50** formulations exhibited less surface peeling compared to those with higher orange peel content. Overall, both volume and density significantly decreased after oven drying.

Table 1. Volume of fungal biocomposite samples in both states wet and dry, volume variation, drying shrinkage (DS) and mass loss in drying process.

Entry	Sample	V_i (dm^3)	V_f (dm^3)	ΔV (dm^3)	DS (%)	Mass Loss (%)
1	**REF**	(1.64 ± 0.02)	(1.31 ± 0.01)	(-0.33 ± 0.02)	(20 ± 2)	(65 ± 3)
2	**O-25**	(1.67 ± 0.02)	(1.20 ± 0.02)	(-0.47 ± 0.03)	(28 ± 2)	(68 ± 3)
3	**O-50**	(1.63 ± 0.01)	(1.05 ± 0.01)	(-0.58 ± 0.03)	(36 ± 2)	(77 ± 1)
4	**O-75**	(1.51 ± 0.02)	(0.88 ± 0.02)	(-0.63 ± 0.03)	(42 ± 3)	(76 ± 5)
5	**O-90**	(1.54 ± 0.01)	(0.75 ± 0.02)	(-0.79 ± 0.04)	(51 ± 3)	(79 ± 3)

2.1.1. Thermal Conductivity

The bulk density and thermal properties of the fungal biocomposite specimens are shown in Table 2. On average, the thermal conductivity values are slightly higher than the reference insulation material, expanded polystyrene (EPS) (Table 2, entry 1). This observation suggests that fungal biocomposites incorporating orange peel exhibit thermal insulation performance within the same order of magnitude as EPS, reinforcing their potential use as insulating materials in the construction industry. Furthermore, the thermal conductivity values for all samples fall within the range defined for insulating materials by IRAM Standard 11601 [38]. Among the biocomposite specimens, those formulated with orange peel additives demonstrated better thermal performance than the lemon peel-based samples. However, the formulation composed solely of shredded cardboard/paper bonded with mycelium exhibited the lowest thermal conductivity overall. This suggests that although citrus peel additions could be confer biorepellent properties, they do not enhance, and potentially diminish, the thermal insulating capacity of the composite. Lastly, the thermal performance of the biocomposite containing different concentrations of orange peel remained relatively consistent across different formulations, as the measured values for samples (Table 2, entries 2 to 5) were statistically equivalent.

Table 2. Bulk density (ρ), thermal and mechanical properties of fungal biocomposites.

Entry	Sample	ρ (kg/m^3)	d (cm)	λ (W/mK)	R (m^2K/W)	σ_{10} (kPa)
1	EPS	(26.0 ± 0.05)	2.5	(0.028 ± 0.002)	89.3	(31 ± 3)
2	**REF**	(259 ± 5)	3.7	(0.035 ± 0.001)	105.7	(50 ± 6)
3	**O-25**	(168 ± 3)	4.3	(0.035 ± 0.002)	122.9	(33 ± 3)
4	**O-50**	(152 ± 4)	4.0	(0.036 ± 0.001)	111.1	(20 ± 1)
5	**O-75**	(181 ± 4)	3.3	(0.029 ± 0.001)	113.8	(7 ± 1)
6	**L-25**	—	4.4	(0.043 ± 0.003)	102.3	(18 ± 2)
7	**L-50**	—	4.3	(0.054 ± 0.004)	79.6	(7 ± 1)

2.1.2. Water Absorption

The water absorption behaviour of the orange peel biocomposites is shown in Figure 5. A typical pattern of rapid initial uptake was observed, consistent with previous findings [39]. After 2 h of immersion, the reference sample made solely from cardboard substrate (**REF**) exhibited a 115% increase in weight. With the addition of a small amount of orange peel (**O-25**), water absorption increased to 140%. In samples with moderate to high orange peel content, **O-50** and **O-75**, the weight gain after 2 h achieved to 156% and 157%, respectively.

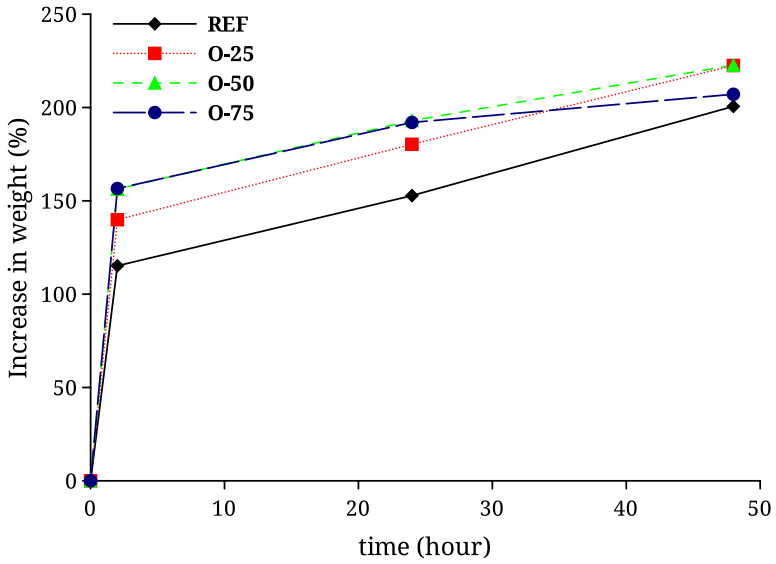


Figure 5. Weight variation of the three formulations of orange peel specimens (**O-25**, **O-50**, **O-75**) and the cardboard/paper specimen (**REF**) by water absorption.

The incorporation of orange peel as a lignocellulosic substrate increased water absorption at early immersion times; however, after prolonged exposure, water uptake across all specimens converged to similar levels, reaching approximately 200% after 48 h. Notably, the water absorption of the biocomposites which contains orange peel was significantly lower than that reported by Appels et al. for fungal biocomposites using rapeseed straw and cotton bur fiber as substrates (ρ = 130 kg/m^3), which showed a weight increase of around 500% after 48 h of immersion [40]. Surprisingly, the **REF** and **O-25** specimens did not suffer catastrophic structural damage even after 24 h of immersion, with the binding capacity of the mycelium still intact, effectively preventing disintegration.

2.1.3. Compression Behaviour

While compressive behaviour is not the most critical property of insulating materials, it remains an important parameter to characterize, as it ensures self-support during service, facilitates handling, and guarantees performance during storage and transportation. For this reason, the compressive behaviour of the fungal biocomposites was assessed by measuring the compressive stress at 10% strain (see Section 3.3.5 for methodological details). Figure 6 shows a typical stress-strain curve obtained from the tests. The shape of the curve corresponds to "example d" as described in the standard [41]. The compressive stress values at 10% strain for each formulation are presented in Table 2.

For the cardboard-based specimen (**REF**), the compressive strength reached 50 kPa (Table 2, entry 2). However, the incorporation of orange peel into the formulation resulted in a progressive decrease in compressive strength. As shown in entries 3, 4, and 5 of Table 2, the values dropped to 33 kPa for **O-25**, 20 kPa for **O-50**, and only 7 kPa for **O-75**. This reduction in mechanical performance is a consequence of the inclusion of large orange peel segments, which introduce substantial voids within the biocomposite structure, weakening its overall integrity. Similarly, formulations containing lemon peel exhibited poor compressive performance, even at low incorporation levels. For instance, **L-25** showed a compressive strength of just 18 kPa, and **L-50** further decreased to 7 kPa (Table 2, entries 6 and 7). It is worth noting that the fungal biocomposites exhibited an extended elastic region. As illustrated in Figure 6, the elastic deformation zone extends up to 20% strain. This behaviour contrasts with the more rigid EPS foams (ρ ~15–25 kg/m^3), which typically

show a more limited elastic region [42], and is instead more comparable to lightweight EPS foams with an bulk density (ρ) of approximately 10 kg/m^3.

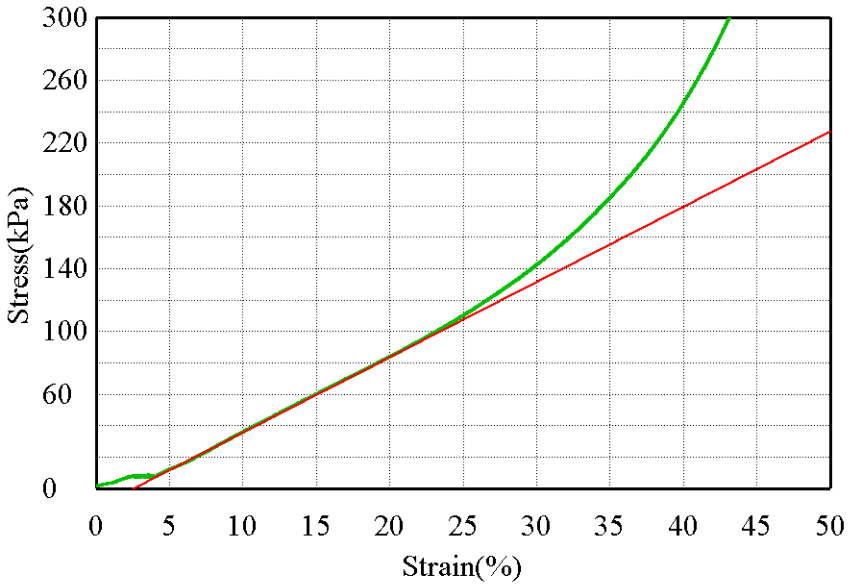


Figure 6. Typical stress vs. strain curve of the fungal biocomposite (green line), the elastic curve is defined to determine the 10% of strain (red line).

2.2. Chemical Properties

2.2.1. Chemical Composition and Morphology

As expected, the chemical composition of the mycelium phase of the fungal biocomposite was as follows: C (26.9%), O (72.1%), Ca (0.5%), Na (0.1%), and K (0.1%), with trace amounts of Al, Si, P, and Mn, in agreement with data reported by Jones et al. [43]. The presence of calcium is attributed to the sanitation process employed in this study, specifically the use of lime in the composite formulation.

In the SEM images shown in Figure 7A,B, the hyphae of the mycelium phase can be observed in the dried state of specimen **O-75**. The measured hyphal diameters ranged from 1.2 to 3.5 µm. Notably, even in the dried state, the fibrous network structure is retained, consistent with observations reported by Jones et al. for the hyphae of *Trametes versicolor* after pyrolysis treatment [43]. This structural persistence is likely due to the presence of chitin in the fungal cell walls. Chitin is a linear polymer composed of the acetylated amino sugar N-acetylglucosamine, which forms microfibrillar structures in biological systems [44,45]. In addition to its mechanical role, chitin contributes thermal stability and flame-retardant properties to the material [46]. These findings are significant, as the integrity of the fibrous microstructure may influence both the porosity and mechanical properties of the resulting fungal biocomposites. Figure 7C displays the characteristic morphology of paper fibers present in the biocomposite. Figure 7D highlights the bonding interface between these fibers and the mycelial hyphae, indicating the role of the fungus as a natural binder. Finally, Figure 7E shows a close mixture of paper fibers and orange peel in close contact with the mycelial hyphae, revealing the hybrid structure of this biocomposite which is composed by trhee phases (cardboard/paper, citrus peel and mycelium).

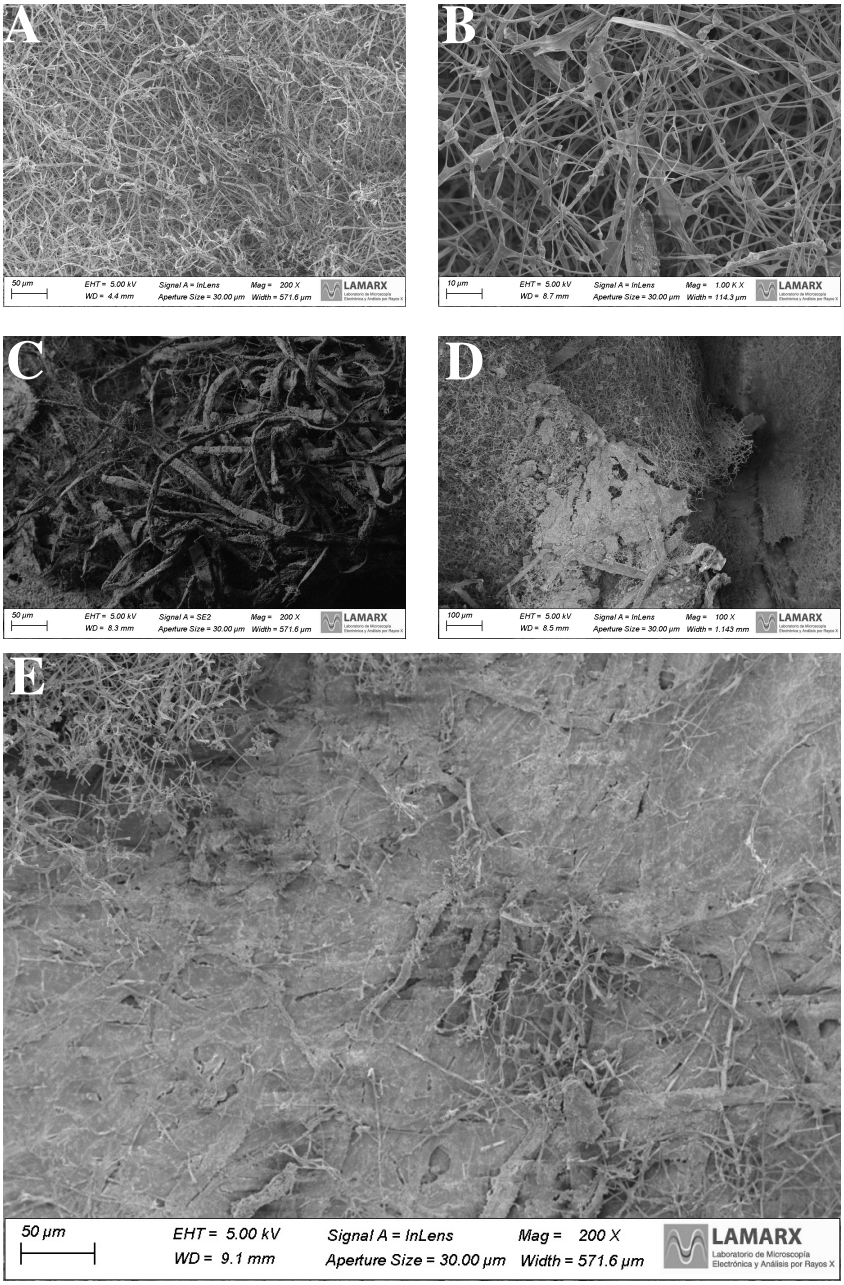


Figure 7. Morphology observed by SEM micrographs of dried sample **O-75**. (**A**,**B**) hyphae of the mycelium, (**C**) morphology of paper fibers, (**D**) bonding interface between paper fibers and the mycelial hyphae and (**E**) mixture of paper fibers and orange peel in close contact with the mycelial hyphae.

2.2.2. Essential Oils

The essential oil profile of the orange peels is presented in the chromatogram shown in Figure 8. As expected, the main volatile component is limonene, in agreement with previous studies [47,48]. In addition, the volatile profile of the dried specimen **O-75**, composed of orange peel, cardboard/paper, and mycelium, was also analyzed. Remarkably, limonene remained the dominant volatile compound, indicating its retention in the composite matrix even after fungal growth and oven drying. The chart in Figure 8 illustrates the relative amount of limonene retained in the final dry biocomposite compared to the fresh state biocomposite. Approximately 20% of the initial limonene content was preserved in the biocomposite. Although this represents a relatively low retention from the raw material, it appears to be sufficient to confer resistance to biodeterioration. This residual limonene

content is associated with the repellent properties of the developed fungal biocomposite, particularly its observed deterrent effect against *T. infestans*.

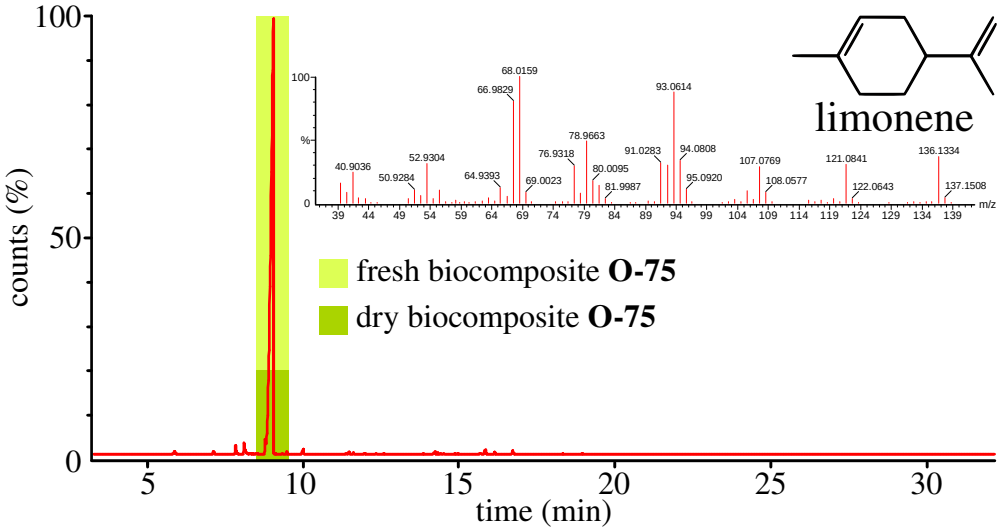


Figure 8. Volatile compounds GC-MS chromatogram profile of fresh fungal biocomposite **O-75** and dried fungal biocomposite **O-75** at 105 °C. The main component is limonene. Relative concentration of this EO marked in colors over the major peak.

2.3. Biorepellent Activity

2.3.1. Sitophilus Zeamais

The repellency of the fungal biocomposite material was evaluated against *Sitophilus zeamais*. The control sample, **REF**, was used as a reference for comparison with the biocomposites **O-75** and **L-75**, which incorporated orange and lemon peels, respectively. The calculated repellency percentage (*SzRP*%) for the biocomposite which contain orange peel was 5.90%, while the specimen which contain lemon peel showed a repellency value of −5.77%. These values are close to zero and, statistically, do not present a significant difference from null repellency. Therefore, it can be concluded that the fungal biocomposites exhibit negligible repellency against *S. zeamais*.

2.3.2. Triatoma Infestans

The biorepellent activity of the biocomposites formulated with varying percentages of lemon and orange peels against stage V nymphs of *T. infestans* is presented in Table 3. The formulations **O-75** and **L-75** (Table 3, entries 5 and 7) exhibited moderate and significant biorepellent effects, corresponding to repellency classes III (40.1 to 60%) and II (20.1 to 40%), respectively, as defined by the repellency classification scale proposed by Talukder and Howse [49]. This moderate bio-repellent activity is likely associated with the high limonene content characteristic of citrus peels. Recent studies have reported that peels of *C. sinensis* (orange) and *C. lemon* (lemon) from citrus-producing regions in northeastern Argentina (Litoral) contain limonene concentrations ranging from 70% to 96% [50]. The retention of this volatile compound, even after fungal growth and drying, may play a crucial role in the repellent efficacy of the resulting biocomposites.

In the analyzed case of dried sample **O-75** the retention of EO is 20% respect to this fresh sample (see Section 2.2.2 and Figure 8). This low relative concentration is sufficient for this material acts as repellent for *T. infestans*. Given its important properties, this material is proposed for use as an insulating building material within the walls or roofs of dry, lightweight construction systems, such as balloon frame. On the other hand, when the proportion of citrus peel decrease, i.e., 50%, the repellent activity against *T. infestans* also

decreases (see Table 3, entries 4 and 6). Taking into account, the future designed material should be prepared with a minimum of 75% of citrus peel to have repellent properties. However, these formulations (**O-75** and **L-75**) exhibit poor mechanical properties (Table 2, entries 5 and 7), which should be addressed by modifying the particle size of the citrus peel to achieve adequate mechanical performance.

Table 3. Repellent activity of fungal biocomposites with different percentages of lemon and orange peels on *Triatoma infestans*.

Entry	Sample	*TiRP*% (%)			Mean [a]	Class [b]
		1 h	24 h	72 h		
1	DEET	(68 ± 33)	(100 ± 0)	(92 ± 18)	87	V
2	**REF**	(−60 ± 28)	(−28 ± 44)	(−36 ± 45)	−41	0
4	**O-50**	(−68 ± 44)	(−44 ± 46)	(−20 ± 80)	−44	0
5	**O-75**	(−20 ± 49)	(68 ± 18)	(92 ± 18)	47	III
6	**L-50**	(−60 ± 18)	(28 ± 44)	(68 ± 33)	12	I
7	**L-75**	(−20 ± 33)	(56 ± 30)	(72 ± 33)	36	II

[a] Average value of repellency in the three times. [b] Repellency class according to scale: Class 0 (0.01 to 0.01%), class I (0.1 to 20%), class II (20.1 to 40%), class III (40.1 to 60%), class IV (60.1 to 80%), and class V (80.1 to 100%).

3. Materials and Methods

3.1. Materials

Cardboard and paper waste: These materials were sourced from packaging and boxes collected as part of source-separated urban waste by residents of Cordoba, Argentina. The containers were subsequently disassembled and shredded using a 4.5 HP mill, producing particles ranging from 0 to 10 mm in size. A mixture of equal parts by weight was used, consisting of 50% *w*/*w* cardboard and 50% *w*/*w* paper. Citrus peel waste: Orange (*Citrus sinensis*) and lemon (*Citrus lemon*) peels were collected in the city of Cordoba, Argentina, during the winter of 2020. Both fruit sources were pesticide-free. Myceliated seed: The spawn of the selected fungal strain, *Pleurotus ostreatus*, was obtained from ProFunga, a local supplier based in Salsipuedes, Cordoba, Argentina. The water used was obtained from the line supply without purification. Medical grade ethyl alcohol (70% *v*/*v*) for sanitation purposes was purchased from Porta Company (Córdoba, Argentina), medical grade hydrogen peroxide (10 volume) for sanitation purposes was purchased from IBC S.R.L., and Blancaley hydrated lime ($Ca(OH)_2$) was obtained from a local supplier. N,N-diethyl-3-methylbenzamide (DEET) was purchased from Sigma-Aldrich (Buenos Aires, Argentina).

3.2. Fungal Biocomposite Production

3.2.1. Pretreatment

We will describe the specimen acquisition process and its graphical representation subsequently in Figure 9. The cardboard waste, crushed in a 4.5 HP mill into particles ranging from 0 to 10 mm, was sterilized in an autoclave at 126 °C for 15 min, then allowed to cool before fungal inoculation. Citrus peels, cut into quarters, were chemically sanitized by immersion in an 8% *w*/*v* calcium hydroxide ($Ca(OH)_2$) suspension for 24 h. This method was chosen to avoid altering the essential oil content due to heat.

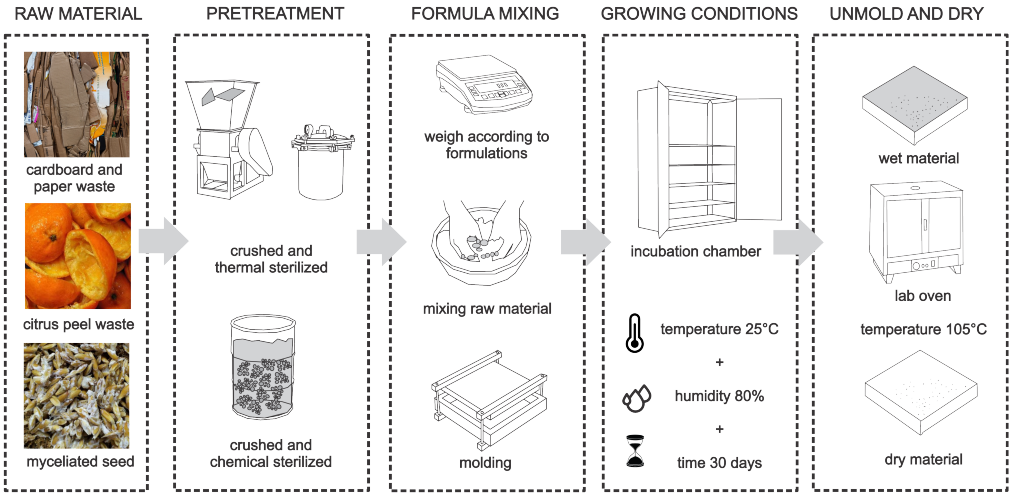


Figure 9. Flowchart of the fungal biocomposite fabrication process.

3.2.2. Sample Formulation

Different quantities of pesticide-free citrus peels, along with sterilized cardboard and paper waste, were mixed with the same proportion of *P. ostreatus* mycelium on oat grain to prepare the fungal biocomposite test specimens. Four different formulations and one reference mixture were designed. The reference formulation (**REF**) did not contain any citrus peels and was composed solely of cardboard and paper waste (90% *w*/*w*) and the selected fungal strain *P. ostreatus* at 10% *w*/*w*. To determine the influence of different citrus peels and their content on the properties of the specimens, the percentage of peel in each formulation was varied. The formulations applied are shown in Table 4. The percentage by weight of orange peel in each formulation was 0% *w*/*w* (**REF**), 25% *w*/*w* (**O-25**), 50% *w*/*w* (**O-50**), 75% *w*/*w* (**O-75**), and 90% *w*/*w* (**O-90**). Similarly, the percentage by weight of lemon peel was also adjusted to 0% *w*/*w* (**REF**), 25% *w*/*w* (**L-25**), 50% *w*/*w* (**L-50**), 75% *w*/*w* (**L-75**), and 90% *w*/*w* (**L-90**). In all cases, the inoculum content of *P. ostreatus* was kept constant at 10% *w*/*w*, and the amounts of paper and cardboard were adjusted to ensure that all specimens had the same final size and mass. All fungal biocomposite test specimens were prepared in duplicate.

Table 4. Formulations expressed as percentages by weight of their components.

Entry	ID	Citrus Peel (%)	Paper/Cardboard (%)	Fungi (%)
1	**REF**	0	90	10
2	**O-25**	25	65	10
3	**O-50**	50	40	10
4	**O-75**	75	15	10
5	**O-90**	90	0	10
6	**L-25**	25	65	10
7	**L-50**	50	40	10
8	**L-75**	75	15	10
9	**L-90**	90	0	10

3.2.3. Growing Conditions

The residues intended to serve as substrates (cardboard, paper and citrus peels) were inoculated with myceliated seeds of the fungus *P. ostreatus*, and the mixtures were placed in molds that had been disinfected with 70% *v*/*v* ethyl alcohol and 10 volume hydrogen peroxide. The different formulations were contained in prismatic wooden molds measuring 35 × 35 × 7 cm, which were lined with aluminum foil to prevent adherence of

the organism, and fitted with 3 mm thick glass lids to allow visual monitoring of fungal growth. The molds were incubated in a growth chamber with controlled environmental conditions, maintained at a constant temperature of 25 °C and 80% relative humidity. The incubation time was 30 days, during which the white mycelial layer completely colonized the surface of the sample without contamination. It is important to note that, to obtain biocomposites, the incubation period should not exceed the point at which the mycelium has fully colonized the surface, in order to prevent the formation of primordia, the first visible sign of fungal fruiting bodies, which could consume the substrate volume for further growth.

3.2.4. Unmold and Dry

Each cultivated prism was demolded and then divided into four parts to obtain specimens measuring 15 × 15 × 5 cm. The samples were then oven-dried at 105 °C until a constant mass was achieved.

3.3. Physical and Mechanical Characterization

3.3.1. Density

To determine the density of the obtained specimens, geometric measurements and mass determinations were performed (using Equations (1) and (2). The following variables were measured: volume (V) in cm^3, dimensions (d) in cm, mass (m) in kg, and density (ρ) in kilograms per cubic meter (kg/m^3).

$$V = d_1 \times d_2 \times d_3 \quad (1)$$

$$\rho = m/V \quad (2)$$

3.3.2. Drying Shrinkage

The following equations and variables were used to determine the drying shrinkage (DS): the initial volume (V_i) corresponds to the volume of the test specimen after incubation, while the final volume (V_f) refers to the volume of the specimen after oven drying. The difference in volumes was calculated using Equation (3), and the percentage reduction relative to the initial volume was determined using Equation (4).

$$\Delta V = V_f - V_i \quad (3)$$

$$DS(\%) = \frac{|\Delta V|}{V_i} \times 100 \quad (4)$$

3.3.3. Thermal Conductivity

For the thermal conductivity test, the IRAM Standard 11559 was followed, applying the guarded hot plate method [51]. This standard establishes the fundamental values and procedures for calculating the thermal properties of components and construction elements under steady-state conditions. It also includes simplified methods for estimating the thermal behaviour of non-homogeneous flat elements. It is important to note that these calculation methods do not account for air infiltration through components, neither solar radiation on surfaces nor through transparent elements. For the determination of thermal conductivity (λ) in W/mK and related steady-state thermal properties, the following formula was used (Equation (5)):

$$\lambda = \frac{(Q \cdot d)}{(A \cdot \Delta T)} \quad (5)$$

where: λ is the thermal conductivity (W/mK); Q is heat flow (corresponds to the electrical power supplied); d is sample thickness; A is a hot plate area; and ΔT is the temperature difference over the sample.

3.3.4. Water Absorption

For the water absorption test, ASTM D570-22 was followed [52]. This standard determines the relative rate of water absorption in plastic materials when immersed in water. The prism-shaped specimens were first oven-dried at 80 °C to eliminate any residual moisture and then weighed using a laboratory balance. Subsequently, the specimens were immersed in distilled water for periods of 2, 24, and 48 h. After each immersion period, the samples were gently dried with absorbent paper to remove surface moisture and then weighed again to determine the amount of water absorbed.

3.3.5. Compressive Behaviour

The specimens were tested under BS EN 826 [41], compressive strength for insulating materials, in a Shimadzu AGS-X 50 kN Universal testing machine, taking the tension at a deformation of 10% of the height. The presented compressive strength values are the average of four determinations of each formulation. The compressive strength (σ_{10}), in kPa was calculated by the following formula (Equation (6)). The compressive strength of seven formulations was characterized.

$$\sigma_{10} = \frac{F_{10}}{A_0} \tag{6}$$

where: F_{10} is the force corresponding to 10% strain, in Newton (N); A_0 is the initial cross-sectional area of the specimen, in square millimeters.

3.4. Chemical Characterization

3.4.1. Composition of the Essential Oils

The amount of EOs were determined in both wet and dry fungal biocomposite. For this propose a qualitative and semi-quantitative analyses of the EOs were performed using a Perkin Elmer Clarus 580 chromatograph-mass spectrometer equipped with a DB5 column (30 m × 0.25 mm, film thickness 0.25 µm; Elite 5 MS Perkin Elmer, Springfield, IL, USA). The temperature of the injector was 200 °C. The oven temperature was programmed as follows: 60 °C for 5 min, ramped up to 170 °C at 4 °C/min, and then raised to 250 °C at 20 °C/min. Helium was used as the carrier gas and the flow rate was maintained at 1 mL/s. The GC/MS interface temperature was 200 °C. Electron impact mode on mass spectrometer was set at 70 eV with a mass scan range of 40–300 atomic mass units (amu). A 10 g sample of the fungal biocomposite was placed into a 100 mL vial with an airtight septum cap and incubated at 60 °C for 30 min, with the solid-phase microextraction fiber (SPME fiber assembly divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 m, 1 cm long from SupelcoLtd., Bellefonte, PA, USA)) exposed throughout the incubation period to sample the headspace. After extraction, adsorbed VOCs were immediately desorbed at 250 °C in the injection port of the GC during 1 min in splitless mode. The identification of EO compounds was based on the comparison of their mass spectrum and KI with those from the NIST-08 Mass Spectral Library (US National Institute of Standards and Technology) and literature data. The amount of each EO constituent was expressed as a relative percentage by peak area normalization.

3.4.2. Chemical Composition

The chemical composition of the mycelium phase of the fungal biocomposite was determined by averaging several energy-dispersive X-ray spectroscopy (EDXS), in a Sigma

Zeiss Field Emission Scanning Electron Microscope (FE-SEM) (Carl Zeiss AG, Oberkochen, Germany). For this analisys, the biocomposite was cuted in specimens of 1 cm × 1 cm × 1 cm.

3.4.3. Morphology

The microscopic fungal biocomposite morphology was observed in a Sigma Zeiss Field Emission Scanning Electron Microscope (FE-SEM). Samples for SEM observations were prepared by the cuting of biocomposite in specimens of 1 cm × 1 cm × 1 cm.

3.5. Biological Characterization

3.5.1. Repellent Activity against *Sitophilus Zeamais*

To evaluate the repellent activity of a novel biocomposite derived from mycelium cultivated on citrus peel against *Sitophilus zeamais*, a two-choice olfactometry assay was conducted following the methodology described by Herrera [53], with minor modifications. Adults of *S. zeamais* Motschulsky (Coleoptera: Curculionidae) were used. Insects approximately two weeks old were selected without sex differentiation. They were reared in plastic containers under controlled conditions of 28 ± 2 °C, 70 ± 5% relative humidity, and continuous darkness, using insecticide-free maize kernels as the food source. For the bioassay, 20 non-sexed adults, previously subjected to a 24-h starvation period, were introduced through a 1 × 1 cm central opening in a glass tube olfactometer. At each end of the tube, two containers were placed: one containing 3 g of the treated material (fungal biocomposite with citrus peel) and the other containing 3 g of the control substrate (fungal biocomposite without citrus peel, **REF**), see Table 4. After 2 h of exposure at 28 ± 2 °C, 60 ± 3% humidity relative humidity, the number of insects present at each end was recorded. The percentage of repellency ($SzRP\%$) was calculated according to the Equation (7):

$$SzRP\% = \frac{(C - T)}{(C + T)} \times 100 \quad (7)$$

where C is the number of insects in the control area and T is the number in the treated area [54]. Each treatment was performed in six replicates and the experiment was repeated three times across different periods. Each insect was used only once.

3.5.2. Repellent Activity against Triatoma Infestans

Bioassays were conducted following the methodology described in [49]. Fifth-instar nymphs of *Triatoma infestans*, provided by the Chagas National Service (Córdoba, Argentina), were used one day after receipt. Filter paper discs (9 cm in diameter) were cut in half. One half of the filter paper disc was covered with 1 g of shredded biocomposite containing different concentrations of citrus peel (see Table 4), while the other half was left empty (untreated). As a control, a separate filter paper disc was covered with 1 g of shredded biocomposite without citrus peel (**REF**) on one half and the other half was left empty (untreated). All filter papers were placed on the floor of a Petri dish. Five starved fifth-instar nymphs of *T. infestans* were released at the center of each Petri dish and maintained under controlled conditions: 4 ± 2 °C, 50 ± 5% relative humidity and and photoperiod of 16 h L/8 h D. Each treatment was performed in quintuplicate. Insect distribution was recorded at 1, 24, 72, and 96 h post-treatment. N,N-diethyl-3-methylbenzamide (DEET) was used as a positive control at 0.5% w/v. Data were analyzed by repeated measures ANOVA and a Bonferroni adjustment was applied for multiple comparisons. We worked with a $p < 0.05$. In the event that the treatments were significant, a one-factor ANOVA was applied and for the posterior analysis the Tukey test was applied. Data were analyzed with the statistical software SPSS 15.0 for Windows. The data were converted into repellency

percentage *TiRP*% by means of the Formula (8) where N_c: is the percentage of individuals present in the control half.

$$TiRP\% = 2 \times (N_c - 50) \quad (8)$$

Positive values indicate repellency and negative values indicate no repellence or attraction. Mean values were categorized according to the following scale: Class 0 (>0.01 to <0.1), I (0.1 to 20), II (20.1 to 40); III (40.1 to 60); IV (60.1 to 80), V (80.1 to 100) according to [49].

4. Conclusions

The main achievement of this work is the development of a tested biocomposite composed of orange peels and mushroom mycelium, demonstrating potential for application as an insulating material in the construction industry. Orange peels were shown to be a suitable substrate for fungal growth, as the presence of essential oils did not inhibit mycelial development. The fungus successfully grew and developed within four weeks following the proposed protocol. This demonstrates that biomass from the citrus industry can be effectively valorised as a substrate for cultivating edible fungi. The resulting mycelium from the citrus-paper mixture acted as an efficient natural binder, enabling the production of fungal biocomposite materials. Regarding the characteristics of the fungal biocomposite: the average dry densities and and thermal conductivities of the test specimens are ranged in the insulating building materials parameters. In terms of drying shrinkage, specimens containing orange peel experienced an important volume reductions, with greater shrinkage observed in formulations with higher citrus content. This property should be carefully considered in the design and molding of construction components. Additionally, in specimens where fragmentation and material loss were observed, it is suggested that further investigation into the effect of citrus particle size on structural integrity may be necessary.

The material developed exhibits environmentally friendly characteristics, as it is manufactured from renewable resources, is entirely bio-based, generates no waste, and can be naturally reabsorbed at the end of its lifecycle. It shows potential for application in lightweight and durable panels for thermal insulation in building enclosures. The next step involves conducting standardized laboratory tests to verify whether the material meets the regulatory requirements for its intended use in construction.

Additionally, the potential repellent activity against *T. infestans* observed in the fungal biocomposite adds significant value, suggesting its use as an innovative strategy for home construction or peridomiciliary applications aimed at preventing, or at least reducing, the transmission of Chagas disease. Moreover, given the presence of limonene in the composite, a broader repellent effect against other insects of human relevance can be anticipated. Ongoing studies are currently being conducted to further evaluation and confirmation of the repellent efficacy of this material.

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