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Aspergillus spp. In woodworking settings: Implications for occupational health and safety

Marta Dias [a,b,*], Margarida Rodriguez [a], Bruna Riesenberger [a], Liliana Marques [a], Elisabete Carolino [a], Magdalena Twarużek [c], Robert Kosicki [c], Lídia Gonçalves [d], Liliana Aranha Caetano [a,d], Ana Alastruey-Izquierdo [e,f], Susana Viegas [a,b], Carla Viegas [a,b]

[a] H&TRC – Health & Technology Research Center, ESSL – Escola Superior de Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisbon, Portugal
[b] NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisbon, Portugal
[c] Kazimierz Wielki University, Faculty of Biological Sciences, Department of Physiology and Toxicology, Chodkiewicza 30, 85–064 Bydgoszcz, Poland
[d] Research Institute for Medicines (iMed.uLisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal
[e] Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda (Madrid), Spain
[f] Center for Biomedical Research in Network in Infectious Diseases (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain

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ABSTRACT

Woodworkers are exposed to several potentially harmful agents, including microorganisms that grow in the wood. The most common fungal species in woodworking environments are *Aspergillus, Penicillium*, and *Cladosporium* spp. with occupational exposure to *Aspergillus* spp. posing a significant respiratory risk. This study aimed to assess exposure to *Aspergillus* spp. in two Portuguese woodworking environments and to perform a thorough analysis of *Aspergillus fumigatus* complex isolates collected from 13 DIY stores and 6 Carpentries in Lisbon Metropolitan Area. Sampling combined active and passive methods to assess microbial contamination. *Aspergillus fumigatus* isolates were analysed for their antifungal susceptibility, resistant mechanisms, mycotoxins production and cytotoxic potential in lung (A459) and liver (HepG2) cell lines. The MAS-100 presented *Aspergillus* sections *Aspergilli* and *Flavi* with the highest prevalence in DIY stores and Carpentries, respectively. A total of 1185 *Aspergillus* spp. were recovered, 270 identified as *Aspergillus fumigatus* sensu stricto growing at 37 °C. None of those isolates was resistant to azoles, 99.07% of them produced gliotoxin and 39.9% of them produced cytotoxic effects in at least one cell line. This study comprehended a multi-approach that considered not only sampling methods but also the laboratory assays to be applied in the *Aspergillus* section *Fumigati* isolates recovered from two different woodworking environments, allowing a complete and robust analysis of this specific environment and species. Overall, the findings indicate that woodworkers are exposed to A. fumigatus isolates with relevant pathogenic traits, despite the absence of azole resistance, underscoring the need for continued environmental and occupational monitoring.

1. Introduction

Woodworkers are constantly exposed to several potentially harmful agents, including allergens, carcinogens, and immunotoxic substances. The exposure to wood derivatives, microorganisms that grow in the wood (fungi and bacteria), as well as the byproducts of these microorganisms, including endotoxins and mycotoxins, are recognized as potential contributors to health issues (Dias et al., 2022; J Dutkiewicz et al., 2001; M Dias et al., 2024). Such exposure can lead to impaired lung function, bronchial hyperresponsiveness, and various health conditions, including organic dust toxic syndrome (ODTS), allergic alveolitis, asthma, chronic bronchitis, rhinitis, mucous membrane irritation (MMI), contact dermatitis, and nasal cancer (Dias et al., 2022; M Dias et al., 2024; CDC, 2020). The most prominent health risks are associated with fungi, which can thrive under suitable conditions on stored wood products, such as planks and chips, often as secondary infections of the wood with known health impacts (J Dutkiewicz et al., 2001).

The most common fungal species in woodworking environments are

* Corresponding author at: ESTeSL – Escola Superior de Tecnologia da Saúde de Lisboa, Av. D. João II, Lote 4.69.01, 1990-096 Lisbon, Portugal.
E-mail address: msf.dias@ensp.unl.pt (M. Dias).

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Aspergillus spp., *Penicillium* spp., and *Cladosporium* spp. (Dias et al., 2022). Out of all three, *Aspergillus* presents a higher importance due to the clinical relevance of some *Aspergillus* sections. Occupational exposure to *Aspergillus* spp. has been well-documented (C Viegas et al., 2017; Sabino et al., 2019) since it poses a significant health concern, as it can lead to various respiratory issues, including allergic reactions, asthma, and hypersensitivity pneumonitis (Sabino et al., 2019).

The small size of its conidia facilitates deep inhalation and colonization of the upper and lower respiratory tracts of exposed individuals (Walsh et al., 2008; C Viegas et al., 2021), and several species including *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aspergillus nidulans* and particularly *Aspergillus fumigatus* or cryptic species within these complexes are considered clinically relevant, with *Aspergillus fumigatus* responsible for nearly 60 % of these cases (Bandres et al., 2026; Dladla et al., 2024). This prevalence is attributed to the small size of its conidia and other virulence factors specific to this section (Sabino, 2021), as well to its thermotolerance, adaptability, and virulence traits (Kwon-Chung and Sugui, 2013).

Since the first reports of clinical azole-resistant *Aspergillus fumigatus* strains in the late 1990s (Abraham et al., 1999; Denning et al., 1997; Jeanvoine et al., 2020), the prevalence of these strains has increased globally. Resistance acquisition occurs through two primary routes: long-term azole therapy and using azole compounds in the environment. Despite their differences, both routes share key prerequisites which include a favorable environment for fungal growth and the presence of azole compounds (Jeanvoine et al., 2020; Verweij et al., 2016). The increasing prevalence of resistant strains has led the World Health Organization (WHO) to classify *Aspergillus fumigatus* as a critical-priority fungal pathogen (WHO, 2022). In addition to the emergence of azole resistance, *Aspergillus fumigatus* have the ability to produce mycotoxins, specifically gliotoxin which is the most common mycotoxin produced by this species (Viegas, 2016) and it is associated with respiratory and hepatic health toxicity (Falcone et al., 2011). Importantly, the presence of a fungal species is not a reliable indicator of mycotoxin presence since not all species/strains produce mycotoxins (C Viegas et al., 2022). Nevertheless, when present, mycotoxins can persist in the environment for extended periods due to their stability under harsh environmental conditions (WHO, 2022; Nezis et al., 2019).

Although *Aspergillus fumigatus* has been documented in several Portuguese occupational environments, including hospitals (C Viegas et al., 2021), the waste industry (C Viegas et al., 2022) and firefighter's headquarters (C Viegas et al., 2021), the woodworking environments remain insufficiently characterized. A review of the available literature shows that existing studies in these environments have primarily focused on describing fungal burden, with limited attention to antifungal resistance, mycotoxin production, or cytotoxicity (Dias et al., 2022). Given the increasing concern regarding azole-resistance in *Aspergillus fumigatus*, and considering that a joint report from several European Union agencies has identified wood-related environments as environmental "hotspots" for resistance selection (Authority (EFSA) EFS, European Centre for Disease Prevention & Control (ECDC), Agency (ECHA) EC, Agency (EEA) EE, Agency (EMA) EM, Centre (JRC) ECJR 2025), together with, to the authors' knowledge, the absence of studies assessing exposure in Portuguese woodworking environments, this study aimed to assess exposure to *Aspergillus* spp. in two such environments and to perform a thorough analysis of *Aspergillus fumigatus* complex isolates by evaluating their antifungal susceptibility and resistance mechanisms as well as their mycotoxin production and cytotoxic potential.

2. Materials and methods

2.1. Woodworking environments characterization

This study is part of an enlarged exploratory study aiming to assess microbial occupational exposure in woodworking environments. Thirteen "Do it Yourself" (DIY) stores and six carpentries located in the Lisbon metropolitan area were assessed between December 2022 and November 2023 (Fig. 1). While in stores it was only possible to conduct one sampling campaign in the cold season, in carpentries it was possible to conduct a seasonality study with a sampling campaign in the warm season and another in the cold season. Microbial occupational exposure was assessed in the wood-cutting area (WCA), before wood-cutting (BWCA), and after wood-cutting (AWCA), as well as in other areas of the DIY stores, such as the wood exhibition area (WEA) and the payment area (PA). On the other hand, in carpentries, it was assessed in the bench zone (BZ), machine zone (MZ), warehouse (W), and office (O). When there was no physical separation between BZ and MZ, sampling was conducted at a single location identified as BZ/MZ (Fig. 1).

2.2. Sampling approaches

2.2.1. Active and passive sampling methods

To assess fungal contamination in both woodworking environments, both active and passive sampling methods were used. Air sampling was conducted using two impaction air samplers, the MAS-100 air sampler (Millipore, Billerica, MA, USA) and the Andersen six-stage cascade impactor. Additionally, the Coriolis μ air sampler (Bertin Technologies, Montigny-le-Bretonneux, France) was used for impingement air sampling to analyze the presence of mycotoxins. In carpentries, due to a technical issue with the Coriolis μ air sampler, it was only possible to use it during the warm season.

Active sampling was performed at all sites in both woodworking environments, with an additional outdoor sample collected using both the MAS-100 and Coriolis μ air samplers as a reference. For personal air sampling, two workers (DIY Stores: one from the WCA and one from WEA; Carpentries: one from BZ and one from MZ) were monitored for two hours using an SKC Button Aerosol Sampler fitted with a 0.8 μm, 25 mm polycarbonate filter, connected to an SKC air sampling pump (Fig. 2).

Regarding passive sampling methods, several were collected, namely, Electrostatic Dust Collectors (EDC), settled dust (SD), filters from vacuumed dust, floor surface swabs, filtering respiratory protective devices (FRPD) and mechanical protection gloves (MPG) used by the workers, and e-cloths (EDCP) aiming to assess the accumulation of microorganisms on workers' clothes (Møller et al., 2022) (Fig. 2). All passive sampling methods were collected in all sampling sites except for the EDCP that was placed in two workers (DIY Stores: one from the WCA and one from WEA; Carpentries: one from BZ and one from MZ) during one work shift (8 h). Since this study is part of an enlarged study, the sampling details are described in previously published studies (M Dias et al., 2024), and they are available in a summarized version, in the supplementary material (Table S1).

2.3. Sample extraction, characterization of viable microbiota and isolation of Aspergillus spp

Details from sample's extraction and the characterization of viable mycobiota (Fig. 2) are described in previously published studies (M Dias et al., 2024), and they are available in a summarized version, in the supplementary material (Table S2).

2.4. Molecular detection of Aspergillus sections

Molecular detection of *Aspergillus* sections *Fumigati, Nidulantes*, and *Circumdati* was performed using Real-Time PCR (qPCR) on all passive samples, except for surface swabs. Fungal DNA was extracted from the samples using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, USA) and analyzed with the CFX-Connect PCR System (Bio-Rad). Primer sequences, TaqMan probes, and amplification conditions are provided in the supplementary material (Tables S3 and S4, respectively). As controls, a non-template control served as the negative

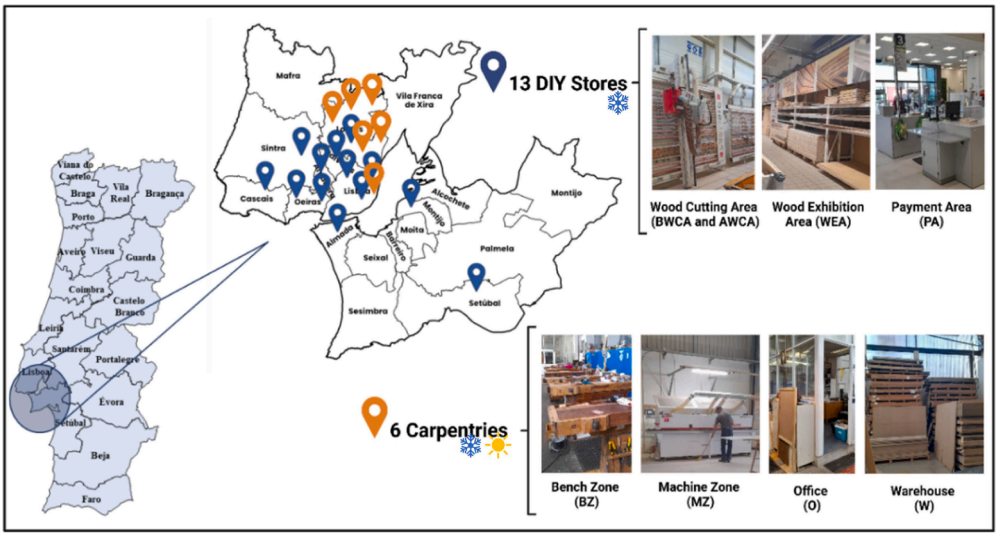

Fig. 1. Geographical distribution of the woodworking companies and workplaces studied.

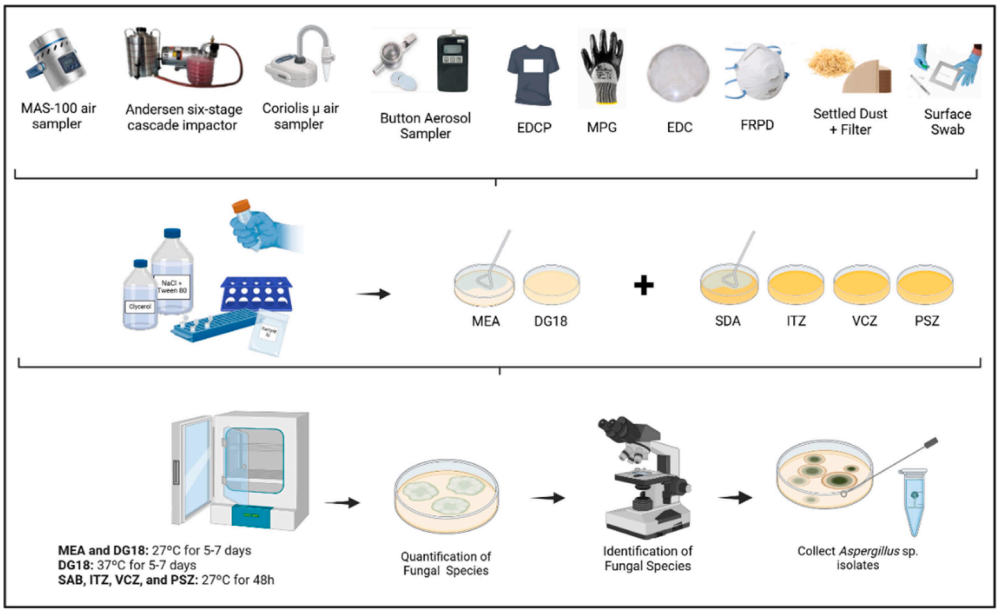

Fig. 2. Sampling and analysis methods for the characterization of viable mycobiota, identification and isolation of *Aspergillus* spp. isolates (Created in BioRender. Dias, M. (2025) https://BioRender.com/j93u416).

control. At the same time, DNA from a reference strain, provided by the Reference Unit for Parasitic and Fungal Infections at the National Health Institute Doctor Ricardo Jorge, IP, was used as the positive control. These reference strains have been sequenced for the ITS, B-tubulin, and Calmodulin genes.

2.5. Analysis of Aspergillus section Fumigati isolates

In this study, it was possible to recover 1185 *Aspergillus* spp. isolates from both woodworking environments, 684 from DIY stores, and 501 from Carpentries. From those isolates, 270 were *Aspergillus fumigatus* sensu stricto (196 from DIY stores, and 74 from Carpentries) that grew in DG18 at 37 °C, therefore considered to have a pathogenic potential and for that reason analyzed aiming to understand the extent of their pathogenicity. The analyzes developed in these isolates go from the testing of their susceptibility to azoles, the detection of resistance mechanisms in Cyp51A, their ability to produce mycotoxins, and also their cytotoxic potential (Fig. 3).

2.5.1. Antifungal susceptibility testing

Antifungal susceptibility of a total of 270 *Aspergillus fumigatus* sensu stricto was determined using the concentration gradient E-test strips method and confirmed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) reference broth microdilution susceptibility testing method (Fig. 4). The procedure details are available in the supplementary material (Methods Supplement S1)

2.5.2. Detection of cyp51A mutations

2.5.2.1. PCR amplification and sequencing. Molecular mechanisms of azole resistance were studied by sequencing the main azole target genes for *Aspergillus fumigatus* sensu stricto (*cyp51A*, including its promoter region). DNA of all isolates was extracted using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, USA). The full coding

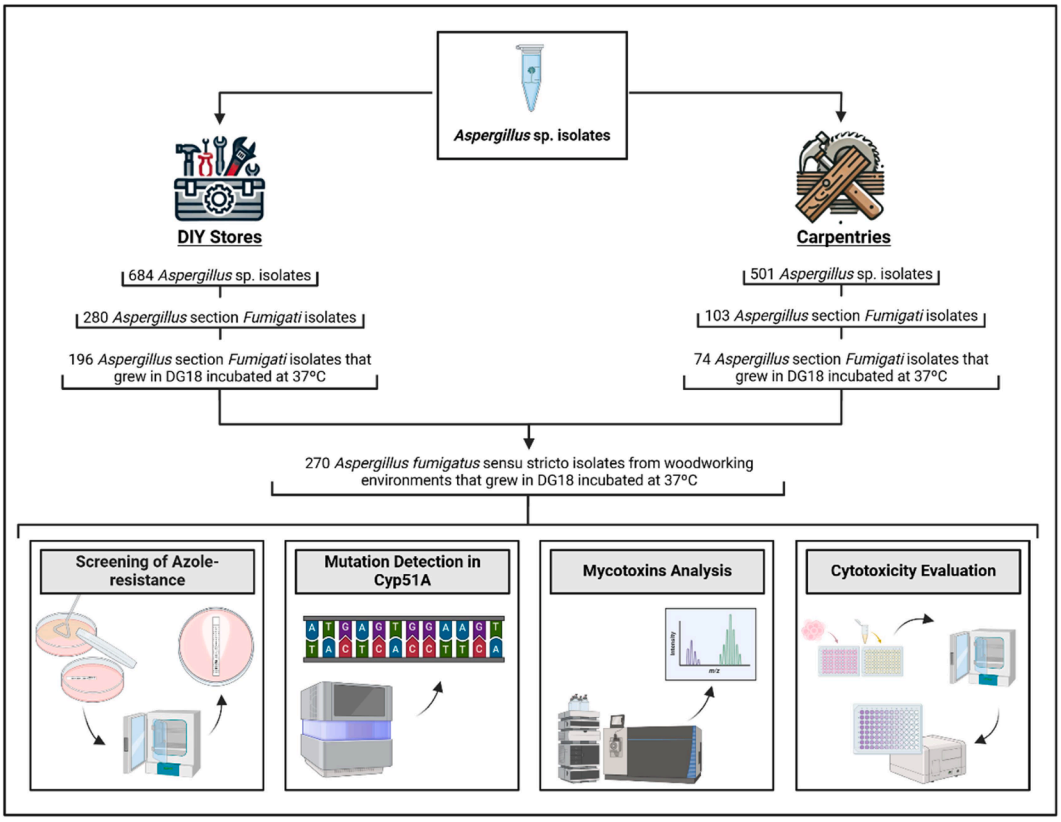

Fig. 3. Analysis applied to *Aspergillus fumigatus* isolates (Created in BioRender. Dias, M. (2025) https://BioRender.com/r99i647).

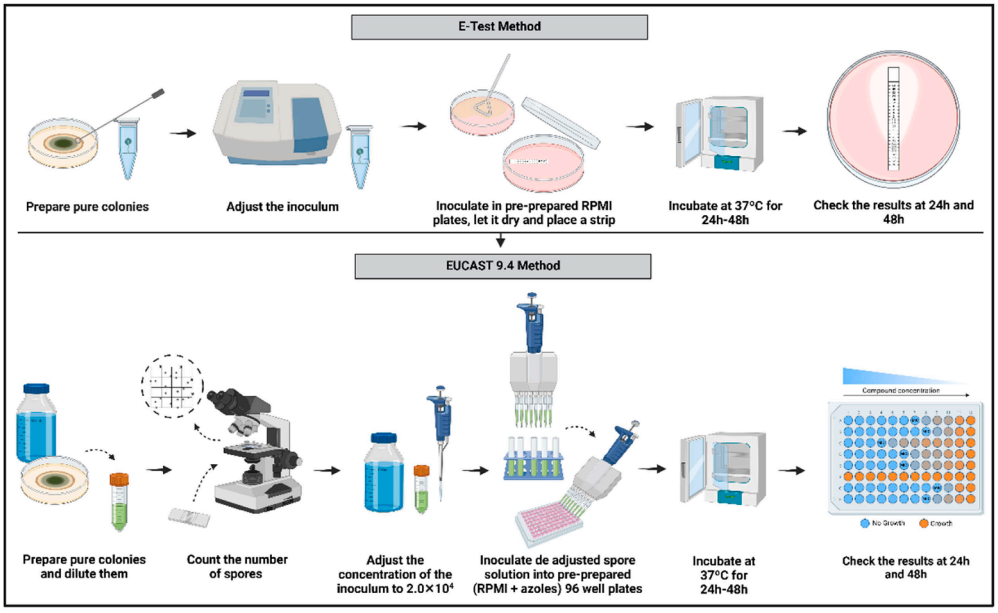

Fig. 4. Antifungal susceptibility testing in *Aspergillus fumigatus* isolates (Created in BioRender. Dias, M. (2025) https://BioRender.com/k47h023).

sequence of *cyp51A* was amplified by using specific primer sets as described previously (Mellado et al., 2007) and are provided in the supplementary material (Tables S5). Negative controls including all PCR constituents but without DNA were included in each amplification run. PCR products were purified using Illustra ExoProStar 1-step technology (GE Healthcare Life Sciences, UK), and subsequently sequenced by Sanger method with an ABI3730XL sequencer (Applied Biosystems, Foster City, CA), as previously described (Diaz-Guerra et al., 2003). DNA sequences were analyzed with SeqMan II software packages (Lasergene; DNAStar, Inc., Madison, WI) and compared with the *cyp51A* sequence of reference strain A1163 of *Aspergillus fumigatus* (NCBI accession number DS499598.1) in order to detect point mutations related to azole resistance.

2.5.3. *Mycotoxin analysis*

In this research, the presence of gliotoxin was carried out on a total of

270 Aspergillus fumigatus sensu stricto isolates. Mycotoxin analysis was performed using a high-performance liquid chromatograph (HPLC) Nexera (Shimadzu, Tokyo, Japan) coupled with a tandem mass spectrometer (MS/MS) 5500 Qtrap (Sciex, Foster City, CA, USA) operating in scheduled multiple reaction monitoring (sMRM) mode. Procedure details, Limit of Detection (LOD), and Limit of Quantification (LOQ) are provided in the supplementary material (Methods Supplement S2). Detailed analytical procedures, including sample preparation, quality assurance and quality control procedures, including method validation parameters and performance characteristics, are described in detail in the Supplementary Material (Methods Supplement S2).

2.5.4. Cytotoxicity evaluation

The cytotoxicity of 193 out of 270 *Aspergillus fumigatus* sensu stricto isolates (only 71.5 % were able to grow as a pure colony) was assessed in human lung epithelial (A549, ATCC® CCL-185™) and human hepatic (HepG2, ATCC® HB-8065) cell lines with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as previously described (Lino et al., 2021; Gaspar et al., 2016), with the details described in the supplementary material (Methods Supplement S3).

2.6. Statistical analysis

Data were analysed using SPSS statistical software version 29.0 for Windows. The results were considered significant at a 5 % significance level. To test the normality of the data, the Shapiro-Wilk test was used. To describe the sample, frequency analysis (n, %) was used for qualitative data and for quantitative data, minimum, maximum, median and interquartile range (Q1 and Q3) were used, as the normality assumption was not verified. To compare the concentration of fungi of the *Aspergillus* sections between two independent groups, the Mann-Whitney test was used and for the comparison between $k > 2$ independent groups, the Kruskal-Wallis test was used since the normality assumption was not verified. In the latter case, when statistically significant differences were detected, the Kruskal-Wallis multiple comparison tests were used.

3. Results

3.1. Aspergillus spp. contamination in woodworking environments

3.1.1. Aspergillus spp. distribution by workplace and sampling method

Considering active sampling methods, in MAS-100, *Aspergillus* section *Aspergilli* (DIY Stores: 64.29 %; Carpentries: 42.11 %) and *Aspergillus* section *Flavi* presented the highest prevalence in malt-extract agar (MEA) and dichloran-dextrose agar (DG18) respectively, in both settings (DIY Stores: 28.03 %; Carpentries: 53.42 %). On BS, *Aspergillus* section *Fumigati* presented the highest prevalence in DG18 (97.12 %) and in DG18 incubated at 37 °C (82.86 %) in DIY Stores. In Carpentries, *Aspergillus* section *Nidulantes* (45.45 %) and *Aspergillus* section *Nigri* (66.67 %) presented the highest prevalence in DG18 in DG18 incubated at 37 °C, respectively (Fig. 5).

Regarding passive sampling, *Aspergillus* section *Nigri* presented the highest prevalence on MEA (DIY stores -> MPG: 100 % | SD: 100 %; Carpentries -> EDC: 100 % | Filters: 100 % | SD: 80 % | Swabs: 50 %). *Aspergillus* section *Aspergilli* presented the highest prevalence on DG18 (DIY stores -> EDC: 63.39 % | EDCP: 56.76 % | Filters: 68.42 % | MPG: 100 % | SD: 77.01 % | Swabs: 45.36 %; Carpentries -> EDCP: 50 %). *Aspergillus* section *Fumigati* presented the highest prevalence on DG18 incubated at 37 °C (DIY stores -> EDC: 91.77 % | EDCP: 64.71 % | Filters: 76.97 % | MPG: 100 % | FRPD: 100 % | SD: 92.64 % | Swabs: 99.33 %; Carpentries -> EDC: 86.67 % | EDCP: 33.33 % | Filters: 46.15 % | SD: 76.67 %) (Fig. 6). On azole supplemented media and considering all sampling methods, no *Aspergillus* spp. were identified.

3.1.2. Aspergillus spp. distribution comparison between seasons in carpentries

Regarding active sampling methods in the warm season, MAS-100 found the highest prevalence of *Aspergillus* section *Nigri* on MEA (100 %) and *Aspergillus* section *Aspergilli* on DG18 (79.07 %). Button Sampler found the highest prevalence of *Aspergillus* section *Fumigati* on MEA (87.50 %), *Aspergillus* section *Aspergilli* on DG18 (71.43 %), and *Aspergillus* section *Nigri* on DG18 37 °C (100 %). A different trend was observed in the cold season, with MAS-100 finding the highest prevalence of *Aspergillus* section *Nigri* on MEA (57.89 %) and *Aspergillus* section *Flavi* on DG18 (53.42 %). Button Sampler found no *Aspergillus* sections on MEA. The highest prevalence of *Aspergillus* section *Nidulantes* on DG18 (45.45 %) and *Aspergillus* section *Nigri* on DG18 37 °C (66.67 %) (Fig. 7).

Concerning passive sampling methods, in the warm season, *Aspergillus* section *Fumigati* presented the highest prevalence on MEA (EDCP: 85.1 % | Filters: 99.0 % | SD: 88.2 % | Swabs: 92.3 %) and on DG18 incubated at 37 °C (EDC: 33.3 % | EDCP: 100 % | Filters: 99.3 % | SD: 94.4 % | Swabs: 98.1 %), whereas *Aspergillus* section *Aspergilli* presented the highest prevalence on DG18 (EDCP: 82.4 % | SD: 81.4 % | Swabs: 42.9 %). In the cold season, *Aspergillus* section *Nigri* presented the highest prevalence on MEA (EDC: 100 % | Filters: 100 % | SD: 80.0 % |

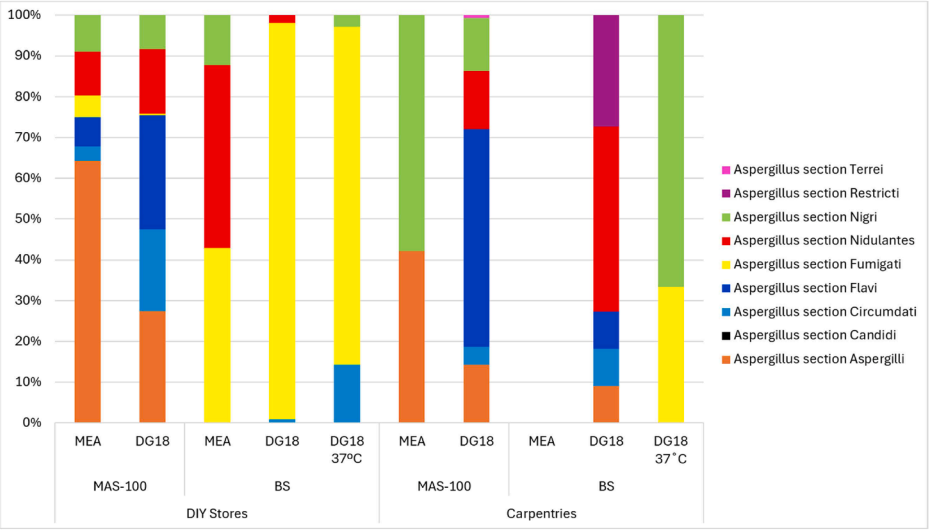

Fig. 5. *Aspergillus* sections distribution with active sampling methods and different medium.

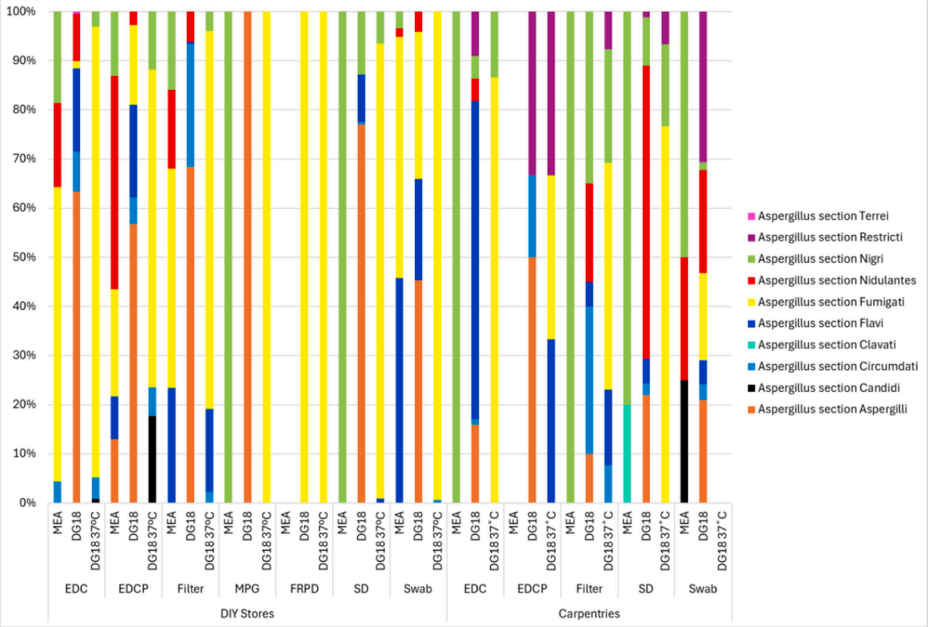

Fig. 6. *Aspergillus* sections distribution in passive sampling methods and different medium.

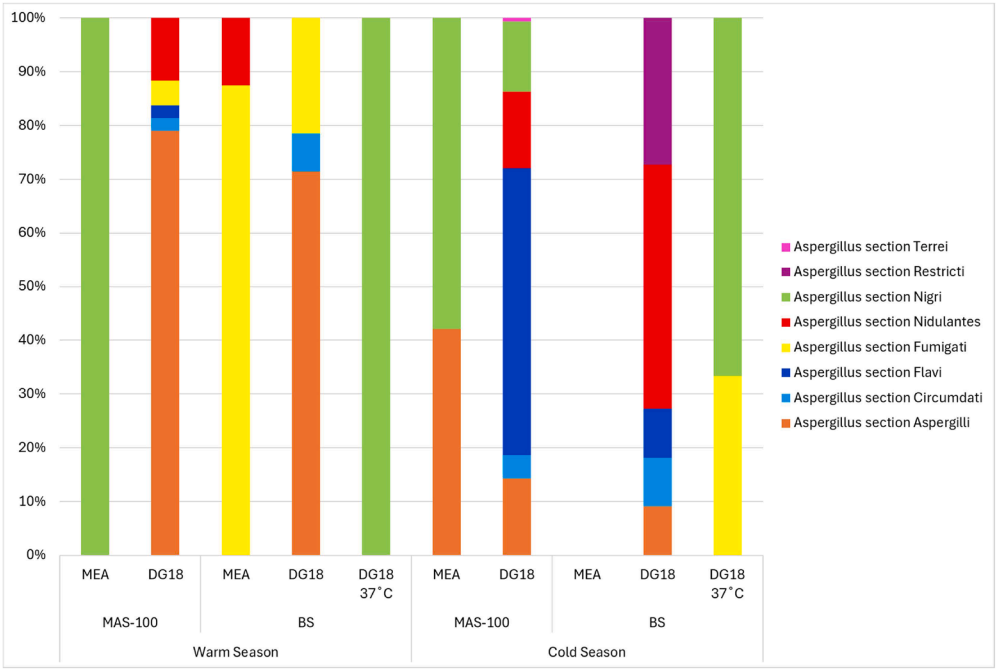

Fig. 7. *Aspergillus* sections distribution between seasons, in active sampling methods from sawmills.

Swabs: 50.0 %), *Aspergillus* section *Flavi* presented the highest prevalence on DG18 (EDC: 64.8 %), and *Aspergillus* section *Fumigati* presented the highest prevalence on DG18 incubated at 37 °C (EDC: 86.7 % | EDCP: 33.3 % | Filters: 46.2 % | SD: 76.7 %) (Fig. 8).

3.1.3. Molecular detection targeting Aspergillus fungal sections

In DIY stores, three *Aspergillus* sections were detected: *Aspergillus* section *Nidulantes* in 7 EDCP (18.42 %), 1 EDC (2.94 %), 5 BS (18.52 %), 6 coriolis (12.25 %), 1 FRPD (20 %), 1 SD (2.56 %) and 5 filters from the vacuum cleaner (10.42 %), *Aspergillus* section *Circumdati* in 1 EDCP (2.63 %), and *Aspergillus* section *Fumigati* in 6 EDC (17.65 %), 24 BS (88.8 %), 48 coriolis (97.96 %), 2 EDCP (5.26 %), 5 FRPD (100 %), 2 MPG (100 %) and 48 filters from the vacuum cleaner (100 %) (Table S3).

In carpentries, two *Aspergillus* sections were detected: *Aspergillus* section *Nidulantes* in 6 SD samples (16.6 %), and in 4 filters from the vacuum cleaner (13.3 %), *Aspergillus* section *Fumigati* in 3 BS (14.29 %), 9 filters from the vacuum cleaner (30 %), and 13 settled dust samples (36.1 %) (Table S6).

3.2. Antifungal susceptibility testing in Aspergillus section Fumigati isolates

3.2.1. E-Test method

The MIC values of the 270 isolates identified as *Aspergillus fumigatus*

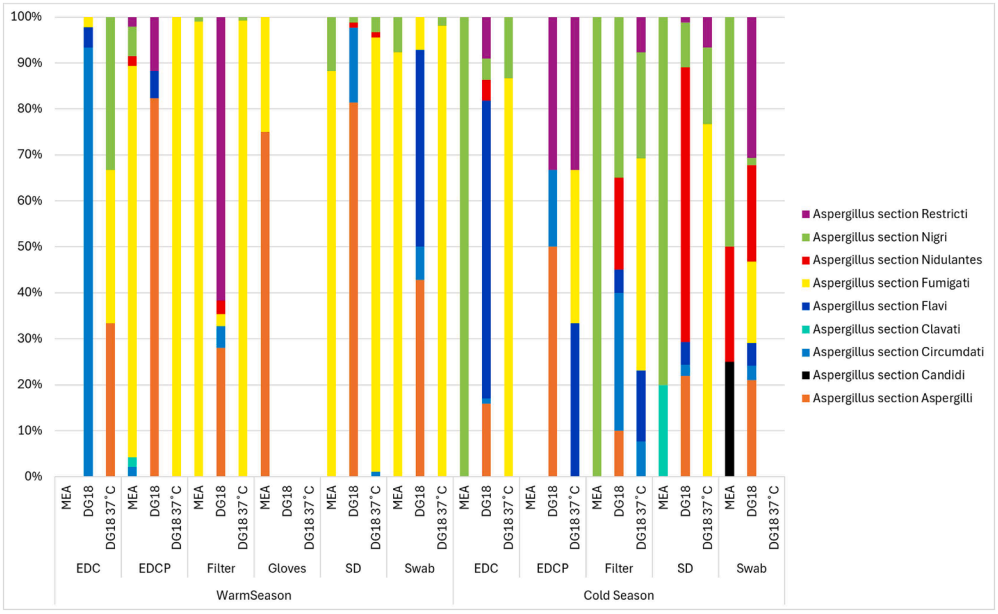

Fig. 8. *Aspergillus* sections distribution between seasons, in passive sampling methods from sawmills.

sensu stricto determined at 48 h with the E-test method are detailed in Table S7. Antifungal resistance was observed in 55 (20.4 %) isolates, with 39 isolates exhibiting resistance to itraconazole, 11 to posaconazole, 3 to amphotericin B, and 2 isolates to both itraconazole and posaconazole.

3.2.2. EUCAST 9.4 method

The 55 isolates that were classified as resistant were tested using the broth microdilution method (EUCAST 9.4) to confirm their susceptibility. The MIC values at 48 h are detailed in Table S8. In this method, 52 isolates (94.5 %) had an MIC below the breakpoint for resistance to all azoles tested and were therefore classified as susceptible. The remaining 3 isolates (5.5 %), all from DIY stores, had an MIC above the breakpoint for resistance to posaconazole and were therefore classified as resistant to posaconazole.

3.3. Mutation detection in cyp51A

To confirm the previous results, *cyp*51A gene of the 55 isolates was sequenced. No mutations were detected in any of the 55 sequenced isolates.

3.4. Mycotoxin analysis

Gliotoxin was detected in all isolates from the DIY stores ($n = 139$; 100 %) with values ranging from 192 μg/kg to 161,000 μg/kg. It was also detected in 75 out of 77 isolates from the carpentries (97.40 %) with values ranging from 902 μg/kg to 137,000 μg/kg.

3.5. Cytotoxicity evaluation

After a 24-hour cell exposure period (in the conditions previously described), 77 out of 193 (39.9 %) isolates induced some cytotoxicity in at least one cell type. Of these, 54 (70.1 %) isolates lowered A549 cell viability to 46.4 % to 70.1 %, and 23 (29.9 %) isolates were cytotoxic to HepG2 cells with cell viability ranging from 42.5 % to 70 %. Noteworthy, 12 out of the 77 (15.6 %) isolates reduced cell viability of both human lung epithelial (A549) and human hepatic (HepG2) cells. Detailed results can be found in supplementary material (Table S9).

3.6. Comparison analysis

For comparison of the *Aspergillus* spp. between stores and carpentry, it was considered only the winter as the stores were not assessed in the summer. For the comparison of the *Aspergillus* spp. between summer and winter it was only considered the carpentries for the same reason. Statistically significant differences were detected regarding the concentration of *Aspergillus* spp. between stores and carpentries in the winter ($U = 21{,}242$, $P < 0.0001$), it was found that the stores have a higher concentration. In carpentries, a significantly higher contamination was detected in the summer ($U = 30{,}356.5$, $P = 0.001$) (Table S10).

From the comparison of the concentration of *Aspergillus* section species between the workplaces, statistically significant differences were detected in the stores ($\chi^2_{K-W}(6)=23.527$, $P < 0.001$), and it was discovered that the CD location differed from the CL locations ($P = 0.030$) and C ($P = 0.007$), showing higher concentration. In DIY stores, no statistically significant differences were detected between workplaces whether in the summer ($\chi^2_{K-W}(6)=8.884$, $p = 0.180$) or in the winter ($\chi^2_{K-W}(5)=8.774$, $P = 0.238$) (Fig. 9).

From the comparison of the concentration of *Aspergillus* section species between stores and carpentry shops, in winter, it was found that the stores presented significantly higher contamination by *Fumigati* ($U = 486$, $P < 0.0001$), by *Nigri* ($U = 777.5$, $P = 0.015$), by *Aspergilli* ($U = 421$, $P < 0.0001$) and by *Flavi* ($U = 343.5$, $P = 0.015$) (Table S11). Comparing the concentration of species from the *Aspergillus* sections, in carpentries, between summer and winter, it was found that summer had a significant higher concentration of *Fumigati* ($U = 860.500$, $P = 0.002$) and *Aspergilli* ($U = 1507.00$, $P = 0.018$) (Table S12).

4. Discussion

Workers who handle or process organic materials, such as woodworkers, are at risk of high fungal particle exposure since occupational environments that involve working with contaminated organic materials are known to have elevated fungal loads (Sabino et al., 2019). Inhalation of large quantities of spores and mycelial fragments can trigger a strong antibody response and lead to respiratory disorders (Dias et al., 2022). The severity of health outcomes depends on the environment's quality, which is influenced by airborne fungal load, specific fungal species or strains, temperature, humidity, ventilation, dust

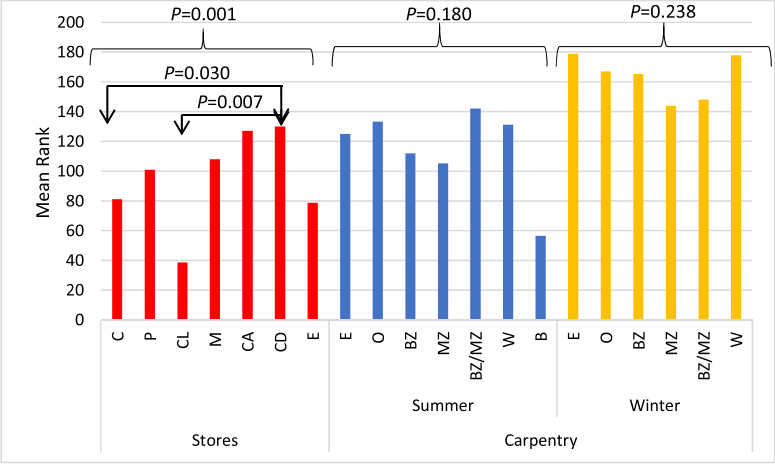

Fig. 9. Comparison of the concentration of species from the *Aspergillus* sections between workplaces, in stores and carpentry. Kruskal-Wallis test results.

and other particles. Poor ventilation, in particular, promotes fungal growth and high spore concentrations, affecting indoor microbiological quality (Sabino et al., 2019). Similar patterns of elevated fungal loads have been reported in Central European (J Dutkiewicz et al., 2001; Klarić et al., 2012; Ljubičić Ćalušić et al., 2013; Rusca et al., 2008), East Asian (Park et al., 2010) and Canadian (Duchaine and Mériaux, 2000) woodworking facilities. Additionally, high concentrations of airborne fungi and recurrent detection of *Aspergillus* spp. were also common in Polish (J Dutkiewicz et al., 2001; Górny et al., 2019) and Croatian (Klarić et al., 2012) woodworking environments, reinforcing the consistency of these exposure patterns across different geographic regions.

Within this broader context, a growing concern in occupational exposure to *Aspergillus* is the emergence of azole-resistant strains in recent decades, with varying prevalence reported worldwide. Most cases of azole-resistant disease are caused by resistant *Aspergillus* section *Fumigati* strains originating from the environment (C Viegas et al., 2017). In this study, several *Aspergillus* section *Fumigati* isolates were recovered from both DIY stores and Carpentries, highlighting the need for further analysis on them. Assessing occupational exposure to microorganisms remains a challenge, and despite the uniqueness of each occupational environment requiring methodological adjustments, occupational exposure to fungi – in this case *Aspergillus* spp. - can be developed using several sampling and analysis approaches. To improve accuracy, a combination of active and passive sampling methods in the sampling campaigns as well as a combination of culture-based methods and molecular tools for the laboratory analysis should be employed for a more comprehensive assessment (Dias et al., 2022), which aligns with the protocol applied in this study.

Considering active sampling methods, *Aspergillus* section *Fumigati* presented a higher prevalence in DIY stores which can be justified by some unique environmental conditions such as the daily customer traffic, the diverse geographic origins of products, ventilation, and cleaning procedures (Hoisington et al., 2016; Nasir and Colbeck, 2010). Additionally, structural conditions such as the lack of physical separation – in most of the stores - between the wood-cutting area and the rest of the store enhances the spread of wood dust particles and consequently the fungal species attached to it. Carpentries presented a higher prevalence of *Aspergillus* section *Fumigati* in passive sampling methods which can also be justified by the unique characteristics of the ones assessed in this study, such as the reduced number of workers and the lack/limited natural or mechanical ventilation which reduces the resuspension of wood particles while enhancing its deposition - as being strictly an occupational environment with the absence of customers and constant production of wood dust, cleaning is not a priority.

The identification of specific work areas with higher fungal loads aligns with international studies, particularly from Norway (Afanou et al., 2018; Straumfors et al., 2020; Straumfors et al., 2019; Straumfors et al., 2018), and Canada (Duchaine et al., 2000), which identify sorting areas and green departments, saw departments, dry timber departments, and debarking as hotspots for fungal exposure. This suggests that specific operational zones in woodworking facilities tend to accumulate higher fungal loads regardless of their geographic location.

The high prevalence of *Aspergillus* section *Fumigati* in DG18 at 37 °C, in both DIY stores and carpentries, allowed us to raise a hypothesis about the pathogenic potential of these isolates. Additionally, although no *Aspergillus* section *Fumigati* isolates were recovered from azole-supplemented media — possibly due to the nutrient competition with fast-growing species such as those from the Mucorales order, which can inhibit the growth of clinically relevant fungi like *Aspergillus* section *Fumigati* (Viegas et al., 2015) — subsequent susceptibility testing showed that, in most cases, they did not grow because they were not resistant strains. It is also important to highlight that a seasonal assessment was developed in carpentries which showed a higher prevalence of *Aspergillus* section *Fumigati* in the warm season. This trend aligns with the impact of seasonality on microbial contamination described in the literature (Afanou et al., 2018; Straumfors et al., 2020; Straumfors et al., 2019; Tirado et al., 2010) since temperature fluctuations and precipitation patterns can directly influence microbial growth, survival, distribution, and susceptibility raising the risk of exposure and infection for woodworkers while presenting significant obstacles and a substantial public health concern (Dias et al., 2022; Frankel et al., 2012).

Only 3 isolates were classified as azole-resistant in this study, although the MICs were just one dilution above the breakpoint for posaconazole and were not resistant to the other azoles nor had mutations in Cyp51A. Despite this low level of resistance, the surveillance of azole resistance in different settings, particularly in those considered “hotspots” for the development of antifungal resistance, is highly recommended for a comprehensive fungal exposure risk characterization. In clinical settings, long-term azole therapy for aspergillosis has contributed to the emergence of azole resistance in *Aspergillus fumigatus* over the past decades (Hagiwara et al., 2016; S Berger et al., 2017). Azole resistance has also been observed in azole-naive patients which may be associated with a second pathway of resistance development, where environmental exposure of *Aspergillus fumigatus* to azole fungicides used in agriculture plays a role (S Berger et al., 2017). Besides these two major areas where azole fungicides are used, there are other significant uses, particularly in the protection of wood. Azoles are widely used as wood preservatives to protect against a variety of wood-destroying fungi, helping to ensure the long-term durability of wood products. First introduced to the wood preservation market in the 1990s, azoles have since become some of the most commonly used fungicides in the wood industry (Derkyi, 2020; Jørgensen and Heick,

2021). The most common azoles used in wood preservation are cyproconazole, propiconazole, and tebuconazole. Additionally, to improve defense against fungi that cause wood to deteriorate, these substances are frequently used with preservatives based on copper, such as copper azole formulations (LN Jørgensen and Heick, 2021; Papola et al., 2025). The use of azole fungicides for wood preservation has significantly reduced the reliance on more hazardous substances (LN Jørgensen and Heick, 2021), however, it has its drawbacks such as the link to the development of azole-resistant *Aspergillus fumigatus* strains.

According to a joint report by European Union agencies, including the European Food Safety Authority (EFSA), the European Centre for Disease Prevention and Control (ECDC), the European Chemicals Agency (ECHA), the European Environment Agency (EEA) and the European Medicines Agency (EMA), wood-related environments, have been identified as environmental "hotspots" for the selection of azole resistance in *Aspergillus fumigatus* (Authority (EFSA) EFS, European Centre for Disease Prevention & Control (ECDC), Agency (ECHA) EC, Agency (EEA) EE, Agency (EMA) EM, Centre (JRC) ECJR 2025). The use of azole-based biocides in wood treatment can contribute to the development of resistant strains of this species, posing a potential risk to human health, especially for immunocompromised individuals (Schoustra et al., 2019). The report highlights the need for appropriate wood waste management and storage practices to prevent and control the spread of azole-resistant *Aspergillus fumigatus* (Authority (EFSA) EFS, European Centre for Disease Prevention & Control (ECDC), Agency (ECHA) EC, Agency (EEA) EE, Agency (EMA) EM, Centre (JRC) ECJR 2025) which presents a serious human health concern since it can compromise the effectiveness of medical azoles used to treat aspergillosis (Berger et al., 2017). It is important to highlight that, although woodworking environments are recognised as potential hotspots for azole-resistance selection (Authority (EFSA) EFS, European Centre for Disease Prevention & Control (ECDC), Agency (ECHA) EC, Agency (EEA) EE, Agency (EMA) EM, Centre (JRC) ECJR 2025), this pattern was not observed in the wood-associated samples analysed in our study.

Azole resistance is known as the ability of fungal strains to withstand azole medication dosages that have good antifungal action in other susceptible isolates. By determining a drug's Minimal Inhibitory Concentration (MIC) against a wide variety of strains, threshold values that differentiate resistant from susceptible strains are established. Different reference methods have been established to characterize *Aspergillus* spp. isolates based on their susceptibility to antifungals, such as those created by the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Arendrup et al., 2012; Hope et al., 2013; MC Arendrup et al., 2016; CLSI 2017; Pérez-Cantero et al., 2020). In this study, we used a commercial method, gradient strips (E-test), the main advantages of using gradient strips include testing a wider range of antifungals and generation of MICs. However, there are disadvantages like its subjective interpretation (Kwizera et al., 2024), and although some studies have compared EUCAST guidelines with E-test and obtained high percentages of categorical agreement (Claudino et al., 2008; Al-Hatmi et al., 2017) another study observed a false resistance in 6 % of the isolates (Marcos-Zambrano et al., 2013). Considering this, the gold standard reference method for antifungal susceptibility testing of yeasts and moulds - broth microdilution - was used to confirm the susceptibility of those 55 isolates following the EUCAST protocol which concluded that 52 *Aspergillus* section *Fumigati* isolates were susceptible to the tested antifungals.

Even so, the same 55 isolates underwent analysis for the detection of azole-resistant mechanisms to allow the confirmation of the antifungal susceptibility tests. Azole resistance in *Aspergillus fumigatus* is primarily driven by mutations in genes involved in the ergosterol biosynthesis pathway, particularly Cyp51A, which encodes the cytochrome P450 14-α-lanosterol demethylase, the main target of azole antifungals (van der Torre et al., 2020; Pontes et al., 2020; Gonçalves et al., 2021). The most common resistance mechanism involves modifications in Cyp51A and its promoter, such as TR34/L98H and TR46/Y121F/T289A, found in environmental and clinical isolates, likely due to extensive agricultural fungicide use (Perlin et al., 2017). Other resistance-associated in *Aspergillus fumigatus* are Cyp51A mutations including substitutions at G54, G138, M220, and G448, with G54 and M220 frequently linked to prolonged azole prophylaxis or therapy (Gonçalves et al., 2021; Perlin et al., 2017), and also a 53-bp tandem repeat has been reported in environmental isolates (Perlin et al., 2017; Le Pape et al., 2016). Any of the 55 isolates from this study showed any azole resistance mechanism in the *cyp*51A gene although 3 of them were resistant to posaconazole.

Although the study was not carried out with the aim of comparing methods, and the E-test was used as a quick and simple method to screen a high number of isolates before testing them with the gold standard method, some discrepancies in the results were found and should be discussed. There are studies discussing the discrepancies between Liofilchem's MIC Test Strip (MTS) method and the EUCAST guidelines (Al-Hatmi et al., 2017; Arendrup et al., 2025; Haldorsen et al.). Arendrup et al. studied the performance of the MIC Strip Isavuconazole Test for susceptibility testing of *Aspergillus fumigatus*. The findings of this study indicated that while the MTS method is practical, there were variations in MIC values when compared to the EUCAST reference method, highlighting potential discrepancies, the importance of evaluating the accuracy of commercial testing methods for fungal pathogens, and the importance of using gold standard methods (MC Arendrup et al., 2016). It is also important to note that both methods have been applied in previous work (Jeanvoine et al., 2017), demonstrating that the combined use of commercial strips and reference protocols is well established in the literature.

Fungi are well known for producing mycotoxins, with major concerns arising from their widespread distribution and toxic effects on both humans and animals (Viegas, 2016). As previously mentioned, gliotoxin is the most common mycotoxin produced by *Aspergillus fumigatus* (Stanzani et al., 2005), which aligns with the results since 97.4 % of the isolates were producing this mycotoxin, and it is associated with pneumotoxic (Fujimoto et al., 2016) and hepatotoxic effects (Wright et al., 2001). It is important to highlight that the high prevalence of gliotoxin does not necessarily correlate with azole resistance in *Aspergillus fumigatus*, which was not detected in most isolates. However, the high prevalence of gliotoxin-producing isolates suggests a potential occupational health concern, as these strains have the capacity to generate biologically active metabolites. Although gliotoxin was not detected in the environmental samples collected (M Dias et al., 2024; Dias et al., 2025), the cytotoxicity observed in several isolates indicates that, under favourable environmental conditions, these metabolites could be produced and potentially contribute to respiratory irritation, epithelial damage, or increased susceptibility to infection (Marcelloni et al., 2023). These findings reinforce the importance of preventive measures and continued monitoring in woodworking environments.

The assessment of the toxicity of microorganisms, and consequently their metabolites, present in occupational environments allows the identification of risk factors for workers' health (C Viegas et al., 2022). In order to develop this assessment, the choice of the right cell lines is crucial to understanding the type of toxicity that can induced in humans, and for that, some aspects have to be taken into consideration such as the characteristics of the species that are being studied, which health conditions are they associated with and also which are the potential effects of the exposure to their mycotoxins. Considering these aspects, and given and that both *Aspergillus fumigatus* and its primary mycotoxin, gliotoxin, have been associated with respiratory (Fujimoto et al., 2016) and hepatic disorders (Falcone et al., 2011; Wright et al., 2001), especially in immunocompromised individuals, in this study, the two cell lines chosen were the human epithelial lung cell line (A549) and the human liver carcinoma cells (HepG2). The results of the study align and highlight the toxicity of this species and consequently, the mycotoxins it produces since around 40 % of them induced toxicity in at least one cell line. This pattern becomes clearer when comparing the proportion of

gliotoxin-producing isolates with the proportion that induced cytotoxicity. Although 97.4 % of the isolates were capable of producing gliotoxin, only around 40 % induced cytotoxicity in A549 or HepG2 cells, suggesting that cytotoxicity may not depend solely on the ability to produce gliotoxin, but also on factors such as the quantity of metabolite produced, strain-specific characteristics, or the presence of additional secondary metabolites. The workplaces with higher fungal concentrations also presented a greater number of toxigenic isolates, which may indicate that environments supporting higher fungal proliferation could also favour species capable of producing bioactive metabolites. Seasonal differences in fungal contamination, including the higher concentrations observed in summer, may reflect the influence of temperature, humidity, and ventilation patterns on fungal growth and aerosolization.

Although the assessment of the cytotoxicity of this particular species is crucial to understanding its potential impact on woodworkers' health, it is equally important to consider the cytotoxic analysis developed in the environmental samples collected – and presented in previous studies (M Dias et al., 2024). The individual assessment of each contaminant is an approach that has been used for both hazard characterization and exposure assessment, often overlooking the health effects of exposure to complex mixtures (Vinggaard et al., 2021; C Viegas et al., 2022). However, occupational settings frequently involve exposure to multiple contaminants simultaneously (Vinggaard et al., 2021; C Viegas et al., 2022; S Viegas et al., 2017; Ladeira et al., 2020), and the toxicity of a mixture may differ significantly from that of each pollutant assessed separately (M Dias et al., 2024).

5. Conclusions

This study comprehended a multi-approach that considered not only sampling methods but also the laboratory assays to be applied in the *Aspergillus* section *Fumigati* isolates recovered from two different woodworking environments allowing a complete and robust analysis of this specific environment and species. A limitation of this study is its cross-sectional design which limits the ability to observe temporal changes in this species' prevalence and resistance development pattern as well as the impact of seasonality, and also the size of the sample and the geographic coverage, which may not fully capture the variability of *Aspergillus* spp. - particularly the prevalence of *Aspergillus* section *Fumigati* - and azole resistance across other woodworking settings. Some concerns were raised, such as (a) the high prevalence of *Aspergillus* section *Fumigati* in both woodworking environments; (b) the higher prevalence of *Aspergillus* section *Fumigati* in the warm season in carpentries; (c) the detection of high levels of gliotoxin; and (d) high levels of cytotoxicity in vitro, in both lung epithelial and human liver cell lines. Overall, these findings allowed us to conclude that woodworkers are indeed exposed to pathogenic fungi and their mycotoxins, and the cytotoxicity results showed us the potential impact in these workers' health. This underscores the need for targeted occupational health strategies in woodworking environments. Integrating measures such as improving ventilation, reducing dust accumulation through regular cleaning and localized extraction, establishing preventive maintenance protocols for wood-cutting areas, ensuring proper storage of wood materials, and implementing routine fungal monitoring programs could help limit fungal proliferation and minimize workers' exposure to toxigenic and potentially cytotoxic fungal species and contribute to more effective management of microbial risks in the woodworking sector. Future research should include a longitudinal assessment in more woodworking settings to track resistance trends over time, assess a more complete impact of seasonal variations, and evaluate the long-term impact of azole fungicide use in woodworking environments.

CRediT authorship contribution statement

Marta Dias: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Margarida Rodriguez:** Writing – original draft, Methodology, Formal analysis. **Bruna Riesenberger:** Writing – original draft, Methodology, Formal analysis. **Liliana Marques:** Writing – original draft, Methodology, Formal analysis. **Elisabete Carolino:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Magdalena Twarużek:** Writing – original draft, Methodology, Formal analysis. **Robert Kosicki:** Writing – original draft, Methodology, Formal analysis. **Lídia Gonçalves:** Writing – original draft, Methodology, Formal analysis. **Liliana Aranha Caetano:** Writing – review & editing, Writing – original draft. **Ana Alastruey-Izquierdo:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Susana Viegas:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Carla Viegas:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, 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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.heha.2026.100173.

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