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A novel nano-sulphur and essential oil-based room freshener

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Resumen

Introducción: *Alternaria* spp y *Candida* spp. son hongos patógenos de ambientes interiores como la casa, la oficina, el aula, etc., causan enfermedades como asma crónica e infecciones sistémicas en individuos inmunodeprimidos a través de la secreción de diversas sustancias tóxicas. Los ambientadores a base de productos químicos disponibles comercialmente que se utilizan para controlar la carga de hongos en el ambiente interior no son beneficiosos para la salud humana. **Objetivo:** proporcionar una alternativa viable en forma de enfoque basado en nanopartículas para el manejo de hongos transmitidos por el aire. **Metodología**: aislamiento, identificación microscópica y bioquímica de hongos de interior; Síntesis de nanopartículas de azufre (SNP) mediadas por *Azadirachta indica*, su detección y caracterización; y evaluación *in vitro* de SNP contra hongos aislados presentes en el ambiente interior. **Resultado:** Los hongos aislados se identificaron como especies de *Alternaria* spp y *Candida* spp. Los SNP mostraron máximos de absorbancia a 291 nm. El análisis NTA mostró un tamaño medio de 188,4 nm y un potencial zeta de -4,94 mV, lo que representa una síntesis de SNP estables. El patrón XRD confirmó la naturaleza cristalina cúbica centrada en la cara de los SNP. El espectro FTIR representó la presencia de compuestos polihidroxilo, nitrilo, ceto, aromáticos y carboxílicos que estabilizaron los SNP. Los ensayos antifúngicos demostraron la actividad significativa de los SNP formulados y del ambientador infundido con aceite de eucalipto. **Conclusión**: Los SNP mediados por *A. indica* se pueden aplicar en la formulación y fabricación de un ambientador ecológico para el manejo de hongos patógenos de interior como *Alternaria* spp y *Candida* spp.

Palabras clave: SNPs, Air freshener, *Alternaria spp., Candida spp*, NTA, FTIR, XRD

Abstract

Introduction: *Alternaria* spp. and *Candida* spp. are the main fungal pathogen of indoor environment like house, office, classroom, etc. These may cause various diseases and infections like systemic infections, or chronic asthma in immunocompromised individuals through secretion of various toxic substances. Chemical-based commercially available room fresheners used to control the fungal load of indoor environment are not beneficial to human health. **Objective:** was to provide viable alternative in the form of nanoparticle-based approach for the management of air-borne fungi. **Methodology:** The present study primarily focuses on the isolation, microscopic and biochemical identification of indoor fungi; *Azadirachta indica*-mediated sulphur nanoparticles (SNPs) synthesis, their detection and characterization; and *in vitro* assessment of SNPs against isolated fungi present in indoor environment. **Result:** The isolated fungi were identified as *Alternaria* spp and *Candida* spp. The SNPs showed absorbance maxima at 291 nm. NTA analysis showed average size of 188.4 nm, and zeta potential of -4.94 mV which represented synthesis of stable SNPs. XRD pattern confirmed the face centered cubic, crystalline nature of SNPs. FTIR spectrum depicted the presence of polyhydroxyl, nitrile, keto, aromatic and carboxylic compounds which stabilized the SNPs. The antifungal assays demonstrated the significant activity of the formulated SNPs and eucalyptus oil infused air freshener. **Conclusion**: It can be concluded that *A. indica*-mediated SNPs can be applied in the formulation and manufacture of an ecofriendly air freshener for the management of indoor fungal pathogens like *Alternaria* spp. and *Candida* spp.

Keywords: SNPs, Air freshener, *Alternaria spp*., *Candida spp*, NTA, FTIR, XRD

1. Introduction

The incidence of fungi in indoor air of residential and official buildings and their management has proven its importance in the field of mycology and has opened new avenues of research (1). Since the last few decades aerobiology is gaining much importance and includes studies of both indoor and outdoor fungi (2). Various studies have reported all around the world including Europe and North America (3, 4). There are numerous fungal species present in the indoor environment like home, offices, classrooms, etc. These includes *Aspergillus* spp. (eg. *A. niger*, *A. versicolor*, *A. flavus*, etc.), *Cladosporium* spp. (*C. cladosporoides*, *C. sphaerospermum*), *Curvularia* spp., *Penicillium* spp. (*P. nigricans*), candida and some yeasts (1, 5-7). These fungi are ubiquitously present where humans spend their most of the time and they cause many diseases and adversely impact the human health. Various diseases caused by the above mentioned fungi are respiratory infections, rheumatologic diseases, immunological diseases and others. The microbial volatile organic compounds (MVOC) from molds produce bronchitis, illness and discomfort when inhaled along with fungal biomass (8, 9). *C. albicans* is responsible for nasal polyps and skin reactivity (10). Fungal spores may cause persistent cough which may lead to asthma in infants (11). Organic dust containing fungal and bacterial spores in non-industrial and non-agricultural area leads to Organic dust toxicity syndrome (12) and hypersensitivity syndrome which shows pneumonia like symptoms (13). Immune diseases caused by molds in damp area include Systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, Sjogren's syndrome and psoriatic arthritis (14). It is estimated that globally nearly 30% buildings have poor quality of indoor rooms and is a result of organic as well as inorganic pollutants originating from both living and non-living materials. The fungal spores also contribute to the living pollutants up to a great extent that enters into indoor environment through ventilation or airing. Usually these are unnoticed but, are responsible for the dangers associated like allergic reactions, mycotoxins and various fungal infections (15, 16).

There is a need to improve the quality of indoor environment. For general purpose commercially available room-fresheners and foggers are used. Most of them are based on the toxic chemicals that smell pleasant but are hazardous to human health (17). Nano-based room fresheners supplemented with essential oils can be a novel and ecofriendly alternative to these toxic air fresheners. In this study, we have evaluated the antifungal activity of air freshener incorporated with *Azadirecta indica* mediated sulphur nanoparticles (SNPs) and Eucalyptus oil (individually and in combination) against the fungi

isolated from the classroom at Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India.

2. Materials and methods

2.1. Isolation and identification of fungi from indoor environment of classroom

The fungal contaminants were isolated from the classroom by open plate exposure method (18). Air samples were collected by exposing Petri plates containing sterilized potato dextrose agar (PDA). The PDA plates were exposed to indoor environment of classroom and kept open for respective time interval (at the height of about 1meter above the floor). The plates were then incubated till the fungal growth is observed. The isolation was done at the height equal to human breathing zone i.e. 1 meter above the floor and the plates were exposed at different locations of classrooms for different time interval. To isolate the appropriate load of fungi the plates were exposed for 15 min and the samples were collected twice a day at around 9 am before entry of students in the classrooms and at around 6.30 pm after leaving the classrooms by all the students. In addition, Petri plates were also exposed to outdoor environment (outside of windows, in corridors, etc.) to know whether fungi are coming from air. After exposure, the plates were incubated at 27±2°C in incubator for 2-3 days. After incubation, the plates were observed for fungal growth. The isolates were cultured in pure form and examined microscopically for their morphological characters.

2.2. Synthesis of Sulphur nanoparticles (SNPs)

The SNPs were biosynthesized by method reported by Awwad et al. (2015) with little modification using the crude extract of *A. indica* leaves as a stabilizing agent and sodium thiosulphate as a precursor molecule (19). The synthesized SNPs were precipitated and purified by ultracentrifugation and washing with sterile distilled water and air dried to form powder.

2.3. Characterization of SNPs

The biosynthesized sulphur NPs were primarily detected using the UV-Vis spectrophotometry by scanning a spectrum for absorbance. Further characterization involved size determination by Nanoparticle Tracking and Analysis (NTA) by NTA LM-20, zeta potential measurement by Zetasizer Nano-ZS 90, FTIR analysis using Bruker's ATR spectrometer and x-ray diffraction pattern was recorded by Rigaku miniflex instrument.

2.4. Extraction of eucalyptus oil

The oil was extracted from *Eucalyptus globulus* by Soxhlet extraction method. The leaves were collected from *Eucalyptus* plant, washed properly in tap water and cut into small pieces. These pieces were added to distilled water inside the assembly. After 4 to 5 hours the oil was extracted and collected in the air tight container.

2.5. Preparation of air freshener

The SNPs-based air freshener was prepared by using the method reported by Trass and colleagues (US patent) (20). The synthesized ingredients were mixed in an appropriate proportion to obtain a stable emulsion of air freshener. These oil in water emulsion contained 88 – 99 % (w/w) of water, about 0.1 - 5% (w/w) of fragrance, 0.1 - 5% (w/w) of surfactant and about 0.1 % by weight preservative with the dispersion preserved against microbial attack. To this emulsion *A. indica* mediated 1% SNPs were added in powder form and sonicated to get uniform distribution of SNPs in emulsion. Further the emulsion was evaluated for its antifungal activity against the isolated *Alternaria* spp.

2.6. Evaluation of antifungal activity of SNPs based air freshener

The *in vitro* antifungal activity was evaluated against the formulated SNPs based air freshener by using Kirby-Bauer disc diffusion method (21) and NCCLS guidelines were followed (22).

3. Results

3.1. Isolation and identification of fungi from indoor environment of classroom

After the exposure and incubation the PDA plates were observed for the visible fungal growth. Various fungal isolates were observed (**Figure 1a**), which were then obtained in a pure form, spores attached to mycelium observed under microscope (**Figure 1b** and **1c** shows spores of Isolate 1) and **Figure 1c** and **1e** shows spores of isolate 2 i.e. selected *Candida* spp.

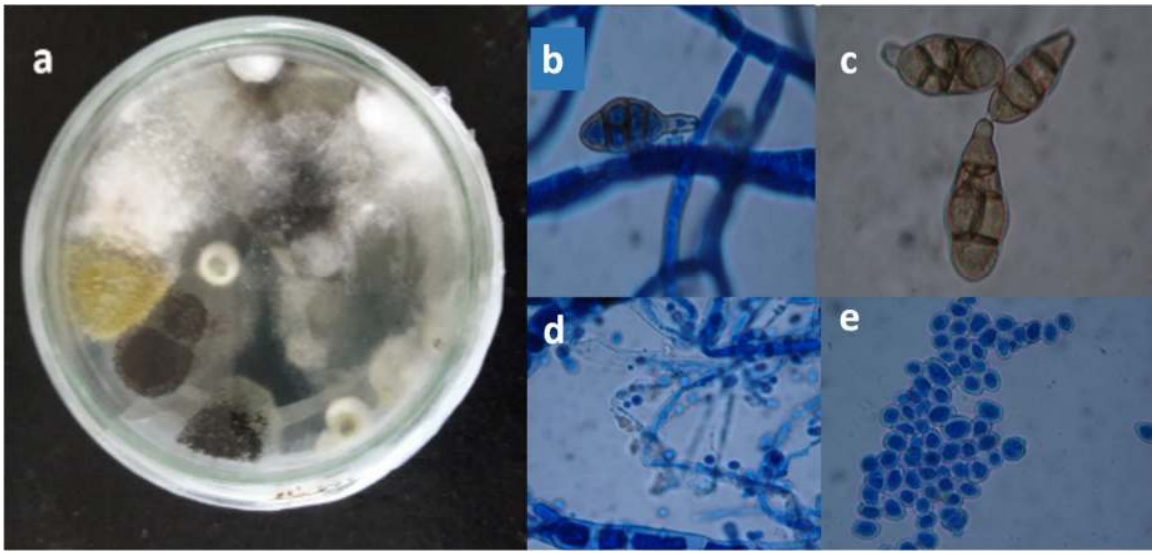

Figure 1. Isolation and microscopic identification of indoor fungi, (a) Visible growth of fungi on PDA plate; (b) Pure culture of fungal isolate 1 i.e. *Alternaria* spp (10X).; (c) Sac like conidia at the end of hyphae (100X); (d) Isolate 2 observed under microscope (10X); (e) Spores of isolate 2 i.e. *Candida* spp. (100X)

3.2. Synthesis and characterization of A. indica- mediated SNPs

The diluted extract of *A. indica* was challenged with sodium thiosulphate and precipitated using strong acid to get turbid, yellowish sulphur nanoparticles at the bottom of reaction mixture (**Figure 2(a)**) (19). The SNPs were extracted and converted to powder form and resuspended into sterile distilled water and were characterized further. The UV-Visible spectrum of SNPs showed absorption peak at 291nm confirming the synthesis of SNPs (**Figure. 2(b)**). The X-ray diffraction pattern of *A. indica* mediated SNPs was studied (**Figure. 2(c)**) to determine the crystalline nature of nanoparticles. The synthesized sulfur nanoparticles showed its characteristic diffraction peak at 24˚, 28˚, 31˚, 35˚, which depicts the presence of facets of FCC (face centered cubic) structure (113), (222), (040),(313), (422), (319). These values are in agreement with JCPDS (Joint Committee on Powder Diffraction, Standard) file no. 34-094.The broad bottom area of the peaks indirectly represents the smaller size of the nanoparticles.

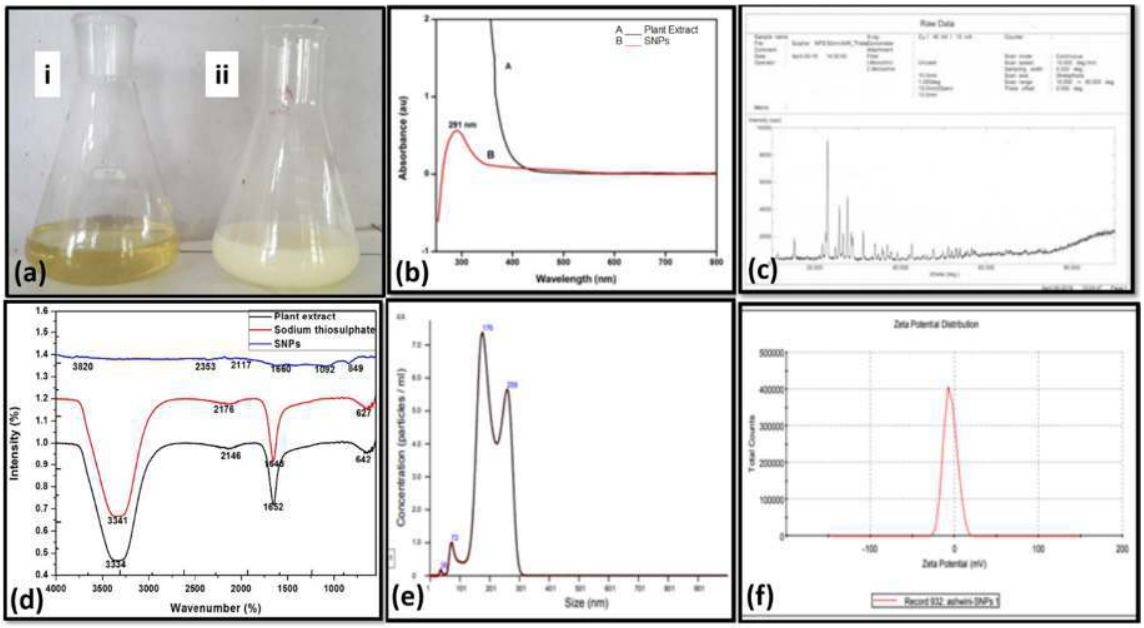

Figure 2. Synthesis and characterization of *A. indica*-mediated Sulphur NPs (a) light-yellow precipitate of SNPs; (b) UV-Vis spectrum of SNPs; (c) XRD pattern of SNPs; (d) FTIR spectrum of SNPs; (e) NTA analysis of SNPs; (f) Zeta potential analysis of SNPs

Fourier transform infrared spectrum of SNPs indicated the presence of peaks present which can be assigned to specific functional groups indicating presence of various functional groups and linkages in the capping layer of SNPs as indicated in the **Table 1**.

Table 1. FTIR spectrum of A. indica-mediated SNPs

Sr. No.	Wavenumber (cm^{-1})	Functional group assigned	Phytochemical present
1	3820	O-H stretch, Hydroxyl group, H-bonded	Poly Hydroxyl compound
2	2353	O-H stretch, Acidic group	Carboxylic acids
3	2117	Multiple bonding	Nitrile compounds
4	1660	C=O stretching vibration, Ketone group	Ketone compounds
5	1092	CN stretch	Aromatic Primary amine
6	849	P-O-C stretch,	Aromatic phosphates

The size determination indicated average size of SNPs was found to be 188.4 nm with standard deviation of 56.06 nm (**Figure 2(e)**). The particle concentration was $5.94x10^7$ particles /ml. NTA analysis revealed that individual nanoparticles and their Brownian motion which can be visually observed and particles by particles size distribution was recorded by Nanoparticle Tracking Analyser LM-20. Zeta potential of SNPs was found to be -4.94 mV as shown in **Figure 2(f)**.

3.2. In vitro assessment of antifungal activity of air freshener against isolated Alternaria and Candida spp.

The eucalyptus oil emulsified air freshener was prepared as per above mentioned method and then was evaluated for the antifungal activity against microscopically identified *Alternaria* and *Candida* spp. *In vitro* antimicrobial susceptibility of the prepared air freshner and its individual components was evaluated on the basis of NCCLS standards and Kirby-Bauer disc diffusion method. The ZOI shown by various components and air freshener are given in **Figure. 3**.

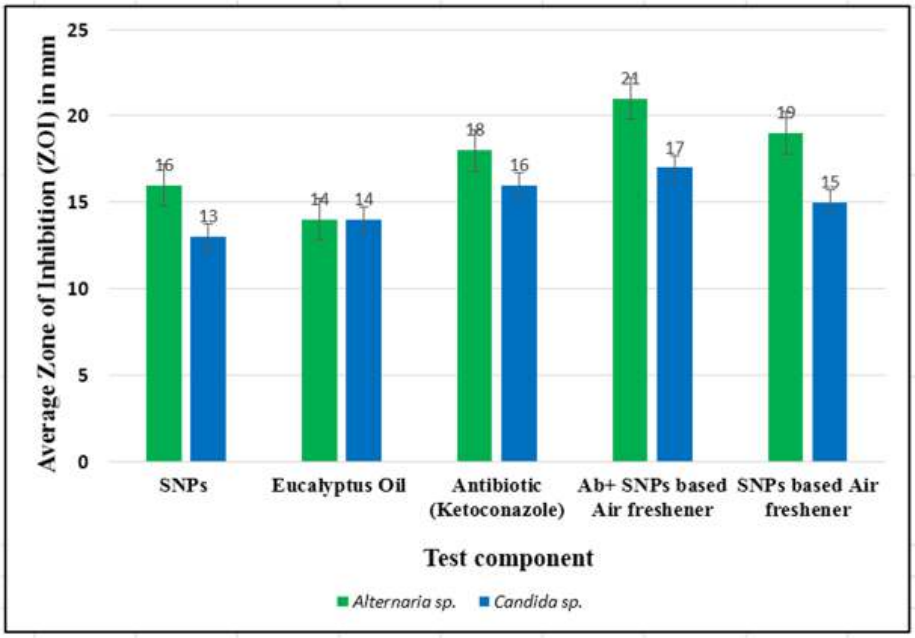

Figure 3. *In vitro* assessment of antifungal activity of air freshener against isolated fungi

4. Discussion

The present study is based on green chemistry principle, which addresses to reduce environmental and health impact of indoor fungi and reduction of chemical usage, to use safe, environmental-friendly methods. The microscopic examination of pure culture showed the presence of sac like dark-brown conidiophore and conidia in chains, belonging to *Alternaria* sp. (23). Other fungi isolated were *Aspergillus* spp., *Fusarium* spp. and *Candida spp*. *Alternaria spp*. are the most common airborne pathogenic fungi present in indoor environment and cause diseases in plants as well as animals in its pa-

thogenesis phase by producing various toxic substances in upper respiratory tract (24) and may cause asthma in immunocompromised individuals. On the basis of morphology of spore and conidia of the isolated fungi, they were identified as *Alternaria* spp. for isolate 1, and *Candida* spp. for isolate 2. The previous reports suggest that the similar types of fungi were identified from the indoor environment of offices, schools and house hold indoors (1, 5, 7).

The synthesis of sulphur NPs was confirmed by light-yellow precipitate (19, 25). Sulphur nanoparticles have been reported to show prominent absorbance spectra in the range of 245–300 nm with maximum absorbance at 280 nm wavelength (26). The values obtained in the present study significantly resemble with the previous reports. The diffraction pattern of sulphur nanoparticles corroborates with the result obtained by Suleiman et al., (27, 28). FCC crystal lattice was predicted from standard JCPDS file (file no. 34-094) for SNPs (25). The FTIR spectrum showed characteristic peaks at 3820, 2353, 2117, 1660, 1092 and 849 cm^{-1} which were assigned to respective functional groups and the corresponding compound as shown in the table no. 1. The peaks and the functional groups are presented in the FTIR spectrum resembles with the previous reports (19). FTIR analysis reveals the presences of aqueous based metabolites from A. indica extract were involved in the stabilization of SNPs.

The **Figure 2(f)**, indicated the stability of the synthesized nanoparticles. Since necked particles with zeta potential more positive than +30 mV or more negative than -30 mV are considered as stable (25, 28). The synthesized particles were found to be stable that may be due to the capping of nanoparticles by biomolecules. The *A. indica*-mediated SNPs, essential oil component (Eucalyptus oil), and standard positive control i.e. antibiotic ketoconazole an *Alternaria spp*. showed antifungal activity in the order as Ab+SNP based air freshener > SNP based air freshener > Ab (ketoconazole) > SNP > Eucalyptus oil. Similarly, the *Candida spp*. showed no significant difference in the antifungal activity of SNPs and eucalyptus oil individually. But in combination with antibiotic ketoconazole, the antimicrobial activity of SNPs was increased up to certain extent. The test components showed the antifungal activity against Candida isolate, in the order shown as, Ab+SNP based air freshener > Ab (ketoconazole) > SNP based air freshener > Eucalyptus oil > SNP. This indicated that there is enhancement of antifungal activity of the formulation in presence of ketoconazole thus indicating the enhanced synergistic activity of nano-based air freshener. The results indicate that *A. indica*-mediated SNPs can be applied for the formulation of an ecofriendly air freshener preceded by thorough evaluation of their activity and compatibility with the human subjects and environmental counterparts.

Bioinspired SNPs were reported to have antibacterial activity (25). Kim and colleagues have reported the chitosan capped antifungal SNPs against *A. flavus* and *C. albican*s (29). The present study indicated that, *A. indica*-mediated SNPs shows significant antifungal activity against the *Alternaria* and *Candida* isolates. Several metal nanoparticles like AgNPs and Copper NPs have already demonstrated activity against pathogens and indoor fungi (30, 31). Our results indicate that, the biological SNPs and essential oil infused air fresheners have potential to inhibit and minimize indoor load of fungi like *Alternaria* and *Candida* spp., by controlling their vegetative growth. Thus, the SNPs based air freshener can be used against the indoor pathogenic fungi and appearance of various diseases inside any building or a classroom can be managed. This indicates that the nano-based air fresheners can be helpful in maintaining sound health of individuals, simultaneously causing minimal damage to the environment.

5. Conclusion

It can be concluded from the given study that the stable SNPs can be synthesized using aqueous leaf extract of *A. indica* having significant antifungal activity. Also these biosynthesized SNPs can be incorporated and applied for the preparation and formulation of an ecofriendly air freshener as a substitute to chemical air fresheners used for domestic purpose. It is still required to thoroughly study and evaluate the formulated product for its pros and cons with respect to the human and environmental health.

References

1. Khan, A.A.H., and Karuppayil, S.M. (2012) Fungal pollution of indoor environments and its management. Saudi J. Biol. Sci., 19(4), 405–426. doi:10.1016/j.sjbs.2012.06.002
2. Mohovic, J., Gambale, W. and Croce, J. (1988) Cutaneous positivity in patients with respiratory allergies to 42 allergenic extracts of airborne fungi isolated in Sao Paulo, Brazil. Immunopathol., 16(6), 397–402.
3. Reynolds, S.J., Streifel, A.J. and McJilton, C.E. (1990) Elevated airborne concentrations of fungi in residential and office environments. Am. Ind. Hyg. Assoc. J., 51(11), 601–604.
4. Şimşekli, Y., Gücin, F. and Asan, A. (1999) Isolation and identification of indoor airborne fungal contaminants of food production facilities and warehouses in Bursa, Turkey. Aerobiologia., 15(3), 225–231. doi:10.1023/a:1007623831010
5. Erkara, I.P., Asan, A., Yilmaz, V., Pehlivan, S. and Okten, S.S. (2008) Airborne Alternaria and Cladosporium species and relationship with meteorological conditions in Eskisehir City, Turkey. Env. Monitor. Assess., 144(1–3), 31–41.
6. Yazicioglu, M., Asan, A., Ones, U., Vatansever, U., Sen, B., Ture, M., Bostancioglu, M. and Pala, O. (2004) Indoor airborne fungal spores and home characteristics in asthmatic children from Edirne region of Turkey. Allerg. immunopath., 32, 197–203.
7. Shirakawa, M.A., Loh, K., John, V.M., Silva, M.E.S. and Gaylarde, C.C. (2011) Biodeterioration of painted mortar surfaces in tropical urban and coastal situations: comparison of four paint formulations. Int. Biodet. Biodeg., 65(5), 669-674.
8. Britton, L.A. (2003) Microbiological threats to health in the home. Clin. Lab. Sci., 16, 10–15.
9. Beezhold, D.H., Green, B.J., Blachere, F.M., Schmechel, D., Weissman, D.N., Velickoff, D., Hogan, M.B. and Wilson, N.W. (2008) Prevalence of allergic sensitization to indoor fungi in 70.West Virginia. Allergy Asthma Proc., 29, 29–34.
10. Burge, H.A., and Rogers, C.A. (2000) Outdoor allergens. Env. Health Persp., 108, 653–659.
11. Bush, R.K. (2008) Indoor allergens, environmental avoidance, and allergic respiratory disease. Allergy Asthma Proc., 29(6), 575-579.
12. Jacobs, R.L., Andrews, C.P., 2003. Hypersensitivity pneumonianonspecific interstitial pneumonia/fibrosis histopathologic presentation: a study in diagnosis and long-term management. Ann. Allergy Asthma Imm., 90, 265–270.
13. Fung, F. and Hughson, W.G. (2003) Health effects of indoor fungal bioaerosol exposure. App. Occup. Env. Hyg., 18, 535-544.
14. Muise, B., Seo, D.C., Blair, E.E. and Applegate, T. (2010) Mold spore penetration through wall service outlets: a pilot study. Env. Monit. Assess., 163(1–4), 95-104.
15. World Health Organization. Indoor air pollutants: exposure and health effects. Report on WHO meeting. WHO Regional Office for Europe: EURO Reports and Studies. 1983; 78: 1-48.
16. Dyląg, M. (2017) Fungi present in home and their impact on human health-A short review. *Insights Biol. Med*., 1, 016-025. DOI: 10.29328/journal.hjbm.1001003
17. https://www.madesafe.org/toxic-chemicals-in-air-fresheners/, 19 January, 2020.
18. Uzochukwu, O.V. and Nkpouto, U. (2013) Airborne fungi in the indoor and outdoor environments of a higher institution in Nigeria. Int. J. Adv. Biol. Res., 3, 9-12.
19. Awwad, A., Salem, N.M. and Abdeen, A.O. (2015) Novel approach for the synthesis of Sulfur nanoparticle using *Alzibia julibrissin* fruit extract. Adv. Mat. Let., 6(5), 432-435.
20. Traas, P.C. and Roehl E.L. (Jan 12, 1988) Process for the preparation of air-freshener gels. Patent No. 4,719,040. United States Patent.
21. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck. M. (1966) Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45, 493-496. BD BBL Prompt Inoculation System Pkg insert #5308-10 Rev 06/2010
22. National Committee for Clinical Laboratory Standards. 2003. Approved standard: M2-A8. Performance standards for antimicrobial disk susceptibility tests, 8th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.

23. Akhtar, K.P., Saleem, M.Y., Asghar, M. and Haq M.A. (2004) New report of *Alternaria alternata* causing leaf blight of tomato in Pakistan. New Dis. Rep., 9. ISSN 2044-0588.
24. Wiest, P., Kurt W., Michael, R.J., Morrissey, A.B., Abelson, T.I., Witt, W., Lederman, M.M. (1987) *Alternaria* Infection in a Patient with Acquired Immunodeficiency Syndrome: Case Report and Review of Invasive *Alternaria* Infections. Rev. Inf. Dis., The University of Chicago Press. 9(4), 799–803. doi:10.1093/clinids/9.4.799. JSTOR 4454171. PMID 3326127.
25. Paralikar, P. and Rai, M. (2017) Bio-inspired synthesis of sulphur nanoparticles using leaf extract of four medicinal plants with special reference to their antibacterial activity, IET Nanobiotech., ISSN 1751-8741, doi: 10.1049/iet-nbt.2017.0079.
26. Chaudhuri, R. G., and Paria, S. (2011). Growth kinetics of sulfur nanoparticles in aqueous surfactant solutions. J. Colloid Interface Sci., 354, 563 –569.
27. Suleiman, M., Al-Masri, M., Ali, A.A., Aref, D., Hussein, A., Saadeddin, I. and Warad, I. (2015) Synthesis of Nano-sized Sulfur Nanoparticles and their Antibacterial Activities. J. Mater. Environ. Sci., 6(2), (2015) 513-518
28. Bramhanwade, K., Shende, S., Bonde, S., Gade, A. and Rai, M. (2015) Fungicidal activity of Cu nanoparticles against *Fusarium* causing crop diseases. Envi Chem. Lett., DOI 10.1007/s10311-015-0543-1
29. Kim, Y. H., Kim, G. H., Yoon, K. S., Shankar, S., & Rhim, J.-W. (2020). Comparative antibacterial and antifungal activities of sulfur nanoparticles capped with chitosan. Microb. Pathogen., 144, 104178. doi:10.1016/j.micpath.2020.104178
30. Ogar, A., Tylko, G., & Turnau, K. (2015). Antifungal properties of silver nanoparticles against indoor mould growth. Sci. Tot. Env., 521-522, 305–314. doi:10.1016/j.scitotenv.2015.03.101
31. Aleksandrowicz-Trzcińska, M., Szaniawski, A., Olchowik, J., & Drozdowski, S. (2018). Effects of copper and silver nanoparticles on growth of selected species of pathogenic and wood-decay fungi *in vitro*. For. Chron., 94(02), 109–116. doi:10.5558/tfc2018-017