

Biomonitoring of airborne fungi and antifungal activity of *Clerodendrum infortunatum* L. against dominant fungi.

Biomonitoreo de hongos aéreos y actividad antifungal de *Clerodendrum infortunatum* L. contra los hongos dominantes

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ABSTRACT

The present study was conducted in Health Centre of Triupra University from August, 2018 to March, 2019 for assessment of aeromycoflora concentration. Burkard personal airsampler and Anderson two stage airsampler were used to detect the nonviable and viable fungal spore respectively. 17 non-viable fungal spores were recorded with the aid of Burkard personal airsampler and 12 viable fungal genera were detected using Anderson two stage airsampler. Higher concentration airborne fungi observed in the month of March. *Aspergillus* sp, Ascospore, Basidiospore, *Curvularia* sp, *Alternaria* sp were found to be *Nigrospora* sp most predominant nonviable fungal genera whereas dominant viable genera were *Aspergillus* sp, *Penicillium* sp, *Cladosporium* sp, *Curvularia* sp, *Trichoderma* sp and *Fusarium* sp in both the environments. The result of antifungal potential of *Clerodendrum infortunatum* showed highest efficacy against *Aspergillus* sp followed by *Penicillium* sp and *Fusarium* sp. This present study provided the baseline information about the viable and non-viable concentration in the study sites. Besides the outcomes of this study along with the insightful explanation could aptly provide basis for strategizing effective preventive measures against airborne-fungi. Those are responsible for causing different agricultural crops diseases and human respiratory ailments. Key words: Anderson two stage airsampler, antifungal potential, Burkard personal airsampler, *Clerodendrum infortunatum*, predominant

RESUMEN

El presente estudio proporciona información de referencia sobre la estimación cuantitativa y cualitativa de aeromicroflora. El muestreador de aire personal Burkard y el muestreador de aire de dos etapas Anderson se usaron para detectar la estimación cuantitativa y cualitativa de aeromicroflora. Se registraron 17 esporas fúngicas no viables con la ayuda del muestreador de aire personal de Burkard y se detectaron 12 géneros fúngicos viables usando el muestreador de aire de dos etapas Anderson. Mayor concentración de hongos en el aire observados en el mes de marzo. *Aspergillus sp*, *Ascospore*, *Basidiospore*, *Curvularia sp*, *Alternaria sp* fueron los géneros de hongos no viables predominantes de *Nigrospora sp*, mientras que los géneros dominantes viables fueron *Aspergillus sp*, *Penicillium sp*, *Cladosporium sp*, *Curvularia sp*, *Trichoderma sp* y *Fusarium sp* en ambos entornos. El resultado del potencial antifúngico de *Clerodendrum infortunatum* mostró la mayor eficacia contra *Aspergillus sp* seguido de *Penicillium sp* y *Fusarium sp*. Este presente estudio proporcionó la información de referencia sobre la concentración viable y no viable en los sitios de estudio. Además, los resultados de este estudio junto con la explicación perspicaz podrían proporcionar una base adecuada para la estrategia de medidas preventivas efectivas contra los hongos en el aire. Esos son responsables de causar diferentes enfermedades de cultivos agrícolas y enfermedades respiratorias humanas.

Palabras clave: muestra de aire Anderson de dos etapas, potencial antifúngico, muestra de aire personal de Burkard, *Clerodendrum infortunatum*, predominante

INTRODUCTION

The ambient air contains different types bio-particulates (pollen grains, insect debris, fungal spores, animal dander, house dust mites, chemicals and food etc.) which cause allergic symptoms (Gravesen, 1979; Shivpuri, 1980). Among these, fungal spores and pollen grains are considered the most major allergens in the air. However, detailed information such as annual and seasonal variations, daily records, of different bio particles is essential for the effective diagnosis and remedial management of these ailments (Singh *et al.*, 1994).

Medical researchers as well as environmentalists given much attention towards airborne due to its direct impact on health, environments and agriculture. Moulds are available in both indoors and outdoors, so it is difficult to conclude where the sensitivity first emerged and response is solely incited by either an indoor or outdoor source (. Generally people spend 90–95% of their time in indoors, so the knowledge on the ecological factors that affect the presence of aeroallergens indoors are essential in understanding the wellbeing impact and potential intervention methods (Sterling *et al.*, 1998). Indoor fungal spore concentration influence by several factors such as air velocity, presence of filtration or ventilation systems, deposition, re-

emission, outdoor concentration, indoor sources, vibration of the surface, colony structure and moisture conditions (Kulmala *et al.*, 1999; Gorny, 2004).

Indoor air quality is an issue of increasing public health consequence (Mentese *et al.*, 2015). Specifically, fungal contamination in indoor environments is responsible for a wide range of unpleasant health effects (Meheust *et al.*, 2014). In addition, fungal contamination in indoor environments has also been related to sick building syndrome (Crook *et al.*, 2010) which includes headache; irritation of the eyes, nose, and throat; lethargy; nausea; dizziness; and chest tightness (Karmakar *et al.*, 2019; Skov, 1992). Curation of indoor environments with fungal contamination is essential for protecting human health¹⁴. This process should involve mould contaminated material should be remove and treating surfaces with antifungal agent which should kill or hamper the growth of fungi and/or fungal spores^(15,16). However, there is increasing concern regarding the use of synthetic chemicals in the home and as such there is increasing interest from consumers for perceived "natural" alternatives (Mehra *et al.*, 2012; Glegg *et al.*, 2007).

The common name of *Clerodendrum infortunatum* is "Bhandira" in Sanskrit, "Bhant" in Hindi and "Bhari chuda" in Bengali found almost all over the India and is popularly known for its medicinal values (Jain, 1991; Kumari *et al.*, 2003). Literature survey revealed leaves and roots of this useful as antiperiodic, vermifuge, antimicrobial activity, laxative and cholagogue (Nguyen *et al.*, 2003). And also in certain skin diseases *Clerodendrum infortunatum* have been identified as potential antifungal treatments, although there are limited studies investigating the use *Clerodendrum infortunatum* against fungi relevant to indoor air quality. The aims of this study were biomonitoring of airborne fungi indoor and outdoor environments and effect of *Clerodendrum infortunatum* extract against the growth of selected dominant genera.

MATERIALS AND METHODS

Health centre of Tripura University (23°45'34"N 91°15'52"E) (Fig.:1) was selected for monitoring of airborne fungal spore in both indoor and outdoor environments from August, 2018 to March, 2019.

Air sampling was conducted simultaneously with the aid of Burkard personal volumetric air sampler and Andersen two stage samplers for monitoring and assessment of non-viable and viable airborne fungal spores concentration respectively. The sample sucked by Burkard sampler directly taken into glass slides mounted with Vaseline and observed under light microscope (Olympus, Model: CX21i) and the result was expressed as (Spores/m³). The viable spores captured by the Andersen two stages sampler were isolated on malt extract agar medium. The concentration of culturable fungi was expressed as (CFU/m³). The isolated fungal genera identified using standard reference manuals (Ellis, 1971; Domsch *et al.*, 1980; Watnabe, 2002).

The leaves of *Clerodendrum infortunatum* L. were collected from Tripura University Campus. The plant specimens were identified and authenticated by Taxonomy and Biodiversity Lab., with the accession no. TUH 2314, Department of Botany, Tripura University, Suryamaninagar.

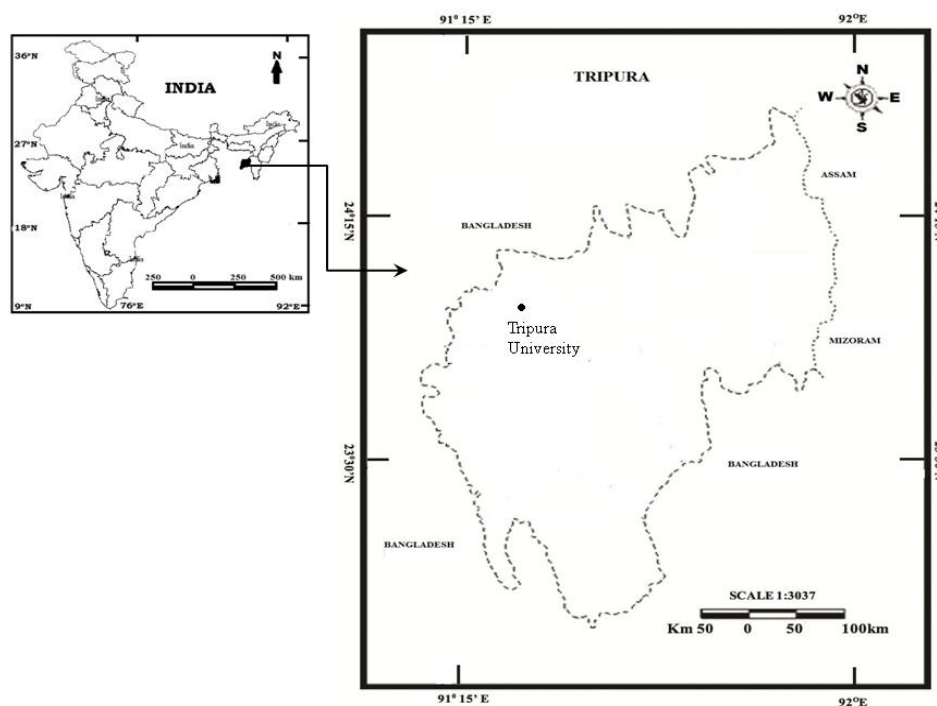


Fig.1: Map showing the sampling site

The selected leaves of the plants were washed with running tap water to remove the dust. The plant materials were dried in room temperature and powdered using mortar and pestle. The plant material was placed in methanol (5g/10ml) and left for forty eight hours with occasional stirrings. The extract was filtered through Whatman filter paper No. 1. The filtrate was evaporated in rotary evaporator at 40°C to get crude extract. The extract was stored in the refrigerator for further use.

The antifungal potential of plants, in terms of inhibition of radial growth of test fungi, was assessed by Poisoned food technique (Mohana *et al.*, 2007). The test fungi were inoculated aseptically at the centre of control (without extract) and MEA (treated with extract) (1mg extract/ml of medium) plates. After incubation, the diameter of fungal colonies in control as well as poisoned plates was measured. Three replications were maintained for each treatment. The diameter of the mycelial growth (mm) of airborne fungi was measured and recorded after 5 days of incubation. Antifungal activity was recorded in terms of inhibition of mycelial growth (%) and calculated using the formula

$$\text{Inhibition of mycelial growth (\%)} = \frac{C - T}{C} \times 100$$

Where 'C' is average diameter of fungal colony in control plates and 'T' is average diameter of fungal colony in poisoned plates (Gupta *et al.*, 2011).

Conversion of colony forming unit and spore per cubic meter of air sampled, percent Contribution, Indoor/Outdoor concentration ratios were calculated using excel 2007 and graph were prepared using Origin 7.

RESULTS

During the sampling period, total 17 fungal genera were identified with the aid of Burkard personnel air-sampler, viz., *Ascospore*, *Aspergillus* sp, *Penicillium* sp, Basidiospore, *Cladosporium* sp., *Curvularia* sp., *Drechslera* sp., *Fusarium* sp., *Lasiodiplodia* sp., *Leptosphaeria* sp., *Nigrospora* sp., *Periconia* sp., *Pithomyces* sp., *Sporidesmium* sp., *Torula* sp., *Agrocybe* sp. and *Trichoderma* sp. Among the 17 genera 13 fungal genera were common in both the environments but 2 fungal genera were in indoor and 2 genera from outdoor were different (Table.1). Comparing of nonviable fungal spore concentration in both environments indoor and outdoor environments it was found that total spore concentration was higher in outdoor environments. The prevalent non-viable mycoflora in outdoor environment were *Aspergillus* sp (350 Spores/m³ and 16.43 % contribution) , *Penicillium* sp (320 Spores/m³ and 15.02 % contribution), *Ascospore* (260 Spores/m³ and 12.21 % contribution), Basidiospore (220 Spores/m³ and 10.33 % contribution), and *Curvularia* sp (140 Spores/m³ and 6.57 % contribution) whereas in indoor environment the recorded prevalent mycoflora were *Aspergillus* sp (100 Spores/m³ and 10.31 % contribution) , *Ascospore* (210 Spores/m³ and 21.65 % contribution), Basidiospore (170 Spores/m³ and 17.53 % contribution), *Alternaria* sp (90 Spores/m³ and 9.28 % contribution) and *Curvularia* sp (90 Spores/m³ and 9.28 % contribution). Among the 17 non-viable fungal spores *Ascospore*, Basidiospore was found in higher concentration in both environments.

In case of viable fungi total 12 fungal genera were isolated from both environments, namely *Aspergillus* sp, *Bipolaris* sp, *Aurobasidium* sp, *Penicillium* sp, *Arthroderma* sp, *Cladosporium* sp, *Curvularia* sp, *Trichoderma* sp, *Fusarium* sp, *Alternaria* sp, *Cochliobolus* sp and *Mucor* sp (Table 1). Among these 15 genera 7 genera were parallel outdoor environments but 5 genera were misplaced in indoor environments. The prevalent viable mycoflora in outdoor environment were *Aspergillus* sp (241.332 cfu/m³ and 25.51 % contribution) , *Penicillium* sp (234.269 cfu/m³ and 24.76 % contribution), *Cladosporium* sp (141.5 cfu/m³ and 14.96 % contribution), *Fusarium* sp (88.264 cfu/m³ and 9.33 % contribution), *Curvularia* sp (71.19 cfu/m³ and 7.52 % contribution) and *Trichoderma* sp (63.598 fu/m³ and 6.72 % contribution) whereas in indoor environment the recorded prevalent viable mycoflora were *Aspergillus* sp

(176.675 cfu/m³ and 25.51 % contribution), *Penicillium* sp(204.943 cfu/m³ and 29.59% contribution), *Cladosporium* sp (63.599 cfu/m³ and 9.18 % contribution), *Fusarium* sp (70.67 cfu/m³ and 10.20 % contribution), *Curvularia* sp (63.599 cfu/m³ and 9.18 % contribution) and *Trichoderma* sp (56.528 cfu/m³ and 8.16 % contribution). Monthly concentration of viable and non viable mycoflora greatly varied. This present study revealed in the month of March the spore concentration was highest followed by September, October and August (Fig.3 and Fig.4) in both viable and non viable spore concentration. Fig.5 and Fig.6 showing the dominant viable and non viable mycoflora in both indoor and outdoor environments.

Table 1: Total concentration and percent contribution of the fungal genera in Health Centre of Tripura University

Fungal genera	Non-Viable (NV)				Viable (V)			
	I(Spores/m ³)	PC(%)	O(Spores/m ³)	PC(%)	I(Cfu/m ³)	PC(%)	O(Cfu/m ³)	PC(%)
<i>Aspergillus</i> sp	100	10.31	350	16.43	176.68	25.51	241.33	25.51
<i>Penicillium</i> sp	50	5.15	320	15.02	204.94	29.59	234.27	24.76
<i>Cladosporium</i> sp	40	4.12	250	11.74	63.60	9.18	141.50	14.96
<i>Trichoderma</i> sp	0	0	60	2.82	56.53	8.16	63.60	6.72
<i>Curvularia</i> sp	90	9.28	140	6.57	63.60	9.18	71.19	7.52
<i>Fusarium</i> sp	30	3.09	40	1.88	70.67	10.20	88.26	9.33
<i>Arthroderma</i> sp	0	0	0	0	35.34	5.10	7.06	0.75
<i>Bipolaris</i> sp	0	0	0	0	0	0	7.07	0.75
<i>Aurobasidium</i> sp	0	0	0	0	0	0	7.07	0.75
<i>Alternaria</i> sp.	0	0	0	0	0	0	21.20	2.24
<i>Cochliobolus</i> sp	0	0	0	0	0	0	7.06	0.75
<i>Mucor</i> sp	0	0	0	0	0	0	21.20	2.24
<i>Sporidesmium</i> sp.	0	0	0	0	14.13	2.04	0	0
<i>Pithomyces</i> sp	0	0	40	1.88	0	0	0	0
<i>Torula</i> sp	30	3.09	50	2.35	0	0	0	0
<i>Nigrospora</i> sp.	90	9.28	110	5.16	0	0	0	0
Basidiospore sp.	170	17.53	220	10.33	0	0	0	0
Ascospore sp	210	21.65	260	12.21	0	0	0	0
<i>Periconia</i> sp.	0	0	120	5.63	0	0	0	0
<i>Alternaria</i> sp.	90	9.28	120	5.63	0	0	0	0
<i>Leptosphaeria</i> sp.		0	10	0.47	0	0	0	0
<i>Agrocybe</i> sp.	10	1.03	0	0	0	0	0	0
<i>Sporidesmium</i> sp.	20	2.06	0	0	0	0	0	0
<i>Dreschlera</i> sp	20	2.06	30	1.41	0	0	0	0
Unidentified	20	2.06	10	0.47	0	0	0	0
Sterile	0	0	0	0	7.06	1.01	35.34	3.73

NV=Non-Viable,V=Viable,I=Indoor,O=Outdoor,PC=Percent Contribution

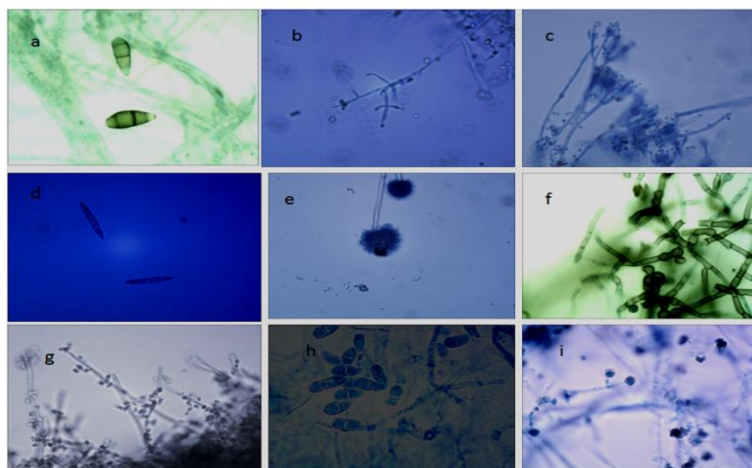


Fig.2: Viable fungal genera, a. *Curvularia* sp, b. *Trichoderma* sp, c. *Penicillium* sp, d. *Fusarium* sp, e. *Aspergillus* sp, f. Sterile dark septate mycelia. *Cladosporium* sp, h. *Bipolaris* sp, and i. Unidentified

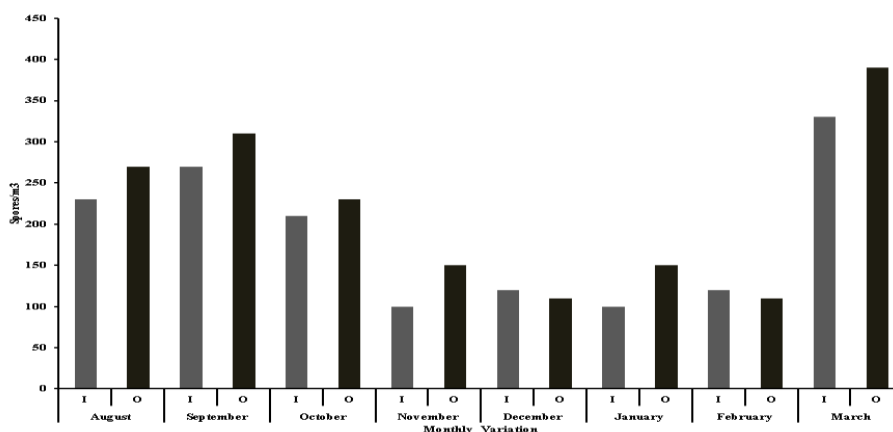


Fig.3: Monthly variation non-viable fungal spores

Results of this study revealed the presence of airborne fungi in the indoor and outdoor air of the Health centre. These results suggested that (i) air of both outdoor and indoor environments were contaminated with airborne fungi, and (ii) the outdoor environments were more contaminated than the indoor environments leading to an exogenous source of indoor contaminations (Table 2).

Table 2: Indoor/Outdoor concentration ratios of airborne fungi isolated from indoor and outdoor air of the Health centre.

Sampled	I/O concentration ratio
Non-viable	0.39
Viable	0.84

Where, I/O concentration ratio greater than (>)1 denotes endogenous source of indoor air contamination whilst I/O ratio less than (<)1 denotes exogenous source of indoor air contamination.

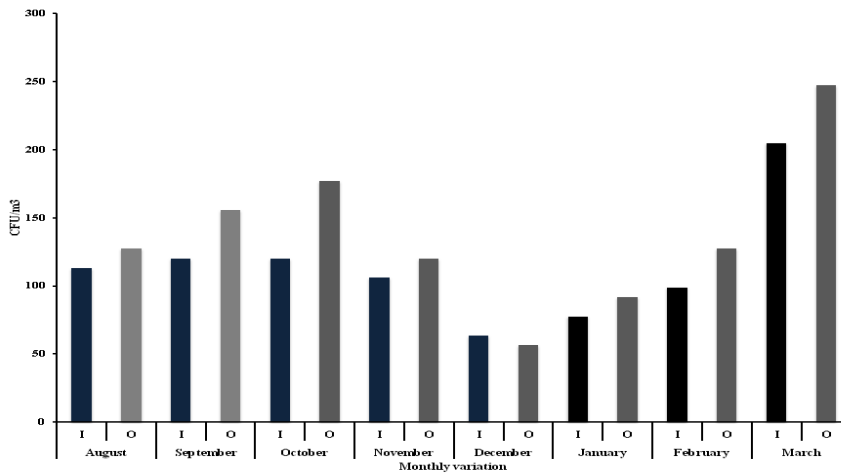


Fig.4: Monthly variation viable fungal spores

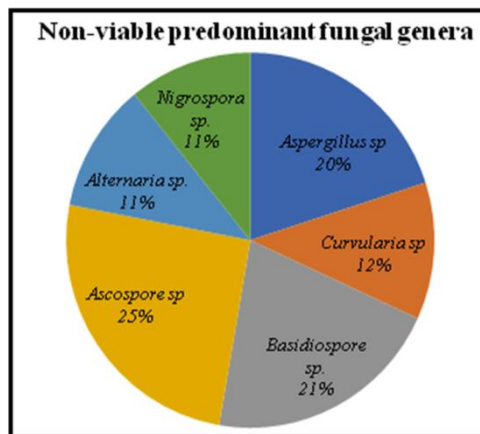


Fig.5: Predominant non-viable fungal genera

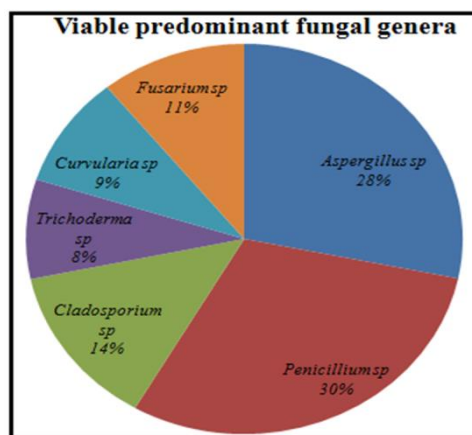


Fig.6: Predominant viable fungal genera

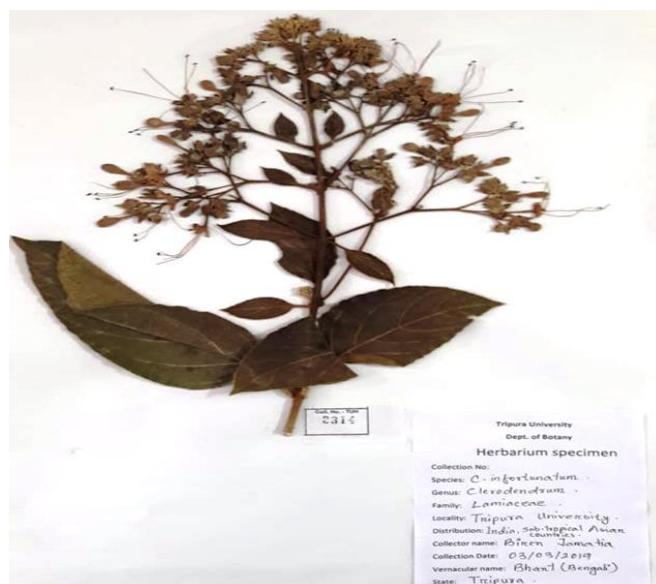


Fig.7: Herbarium of *Clerodendrum infortunatum* L. (TUH 2314)

In the present study, we evaluated the antifungal activity of *Clerodendrum infortunatum* L. (Fig. 7) against 6 airborne-borne fungi by poisoned food technique. Poisoned food technique is one of the most widely used in vitro antifungal assays being used by several researchers to screen the antifungal effect of plants. The six fungal species identified were *Aspergillus* sp, *Penicillium* sp, *Cladosporium* sp, *Curvularia* sp, *Trichoderma* sp, and *Fusarium* sp(Fig.9). These represent the most common environmental saprophytes and human allergen encountered in indoor and outdoor air. The result of antifungal potential of extracts from selected plants is shown in Fig. 8, Fig.9 and Fig.10. The highest efficacy of *Clerodendrum infortunatum* L. extract (more than 80% growth inhibition) were against *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp. The highest growth inhibition ((more than 70% growth inhibition) was against *Cladosporium* sp followed by *Trichoderma* sp (29.41%). On contrast, the lowest efficacy (19.6%) was against *Curvularia* sp (Fig.11).

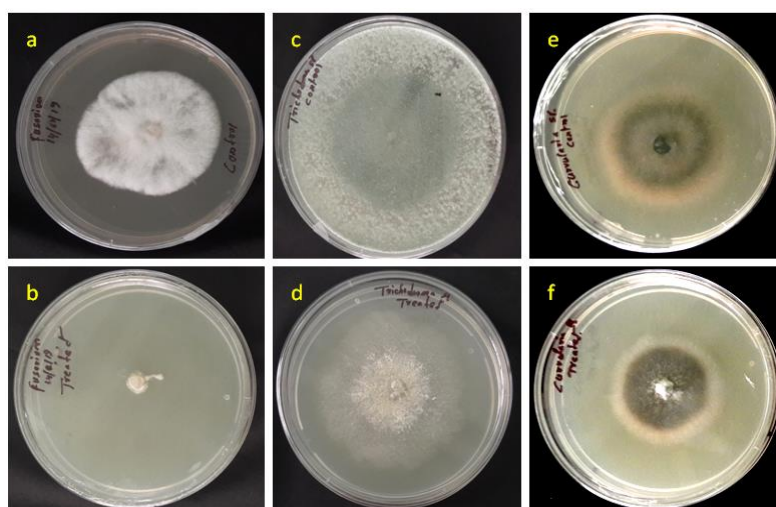


Fig:8: Antifungal activity of *Clerodendrum infortunatum* L. against *Fusarium* sp(a.control,b.treated),*Trichoderma* sp (c.control,d.treated),*Curvularia* sp (e.control,f.treated)

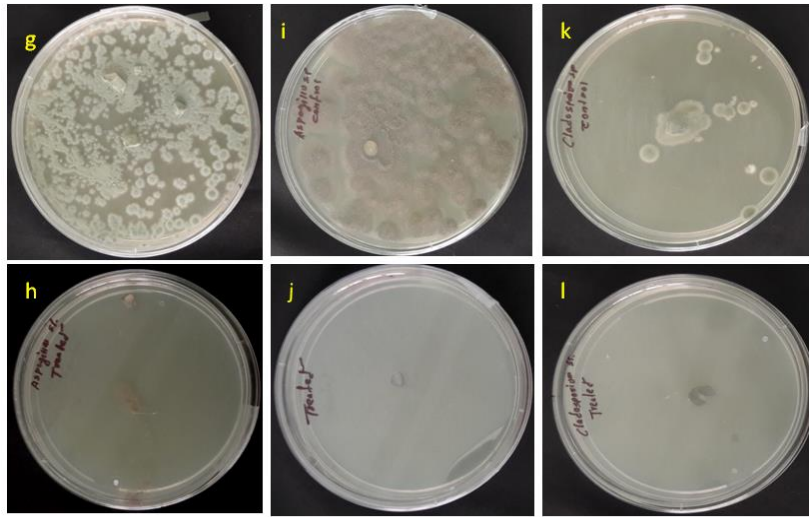


Fig.9: Antifungal activity of *Clerodendrum infortunatum* L. against *Penicillium* sp (g.control,h.treated),*Aspergillus* sp (i.control,j.treated),*Cladosporium* sp (e.control,f.treated)

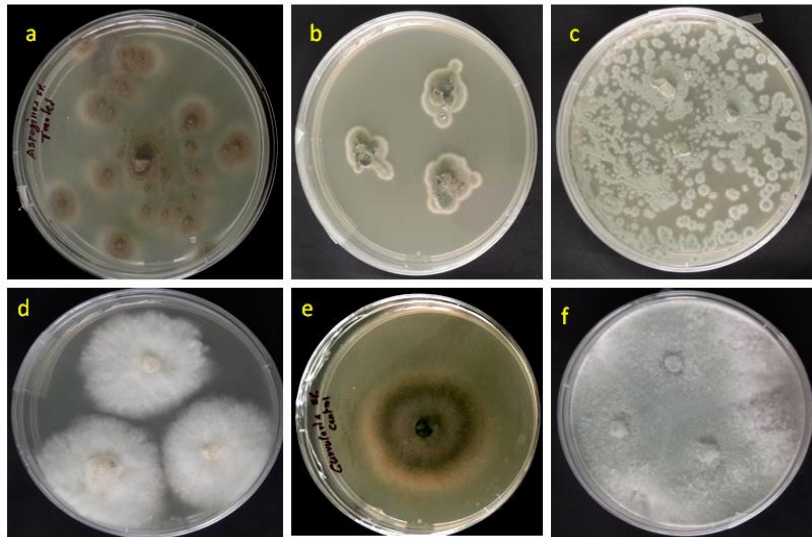


Fig.10: Culture morphology of dominant fungal genera, a.*Aspergillus* sp, b.*Cladosporium* sp,c.*Penicillium* sp,d. *Fusarium* sp, e.*Curvularia* sp and f.*Trichoderma* sp

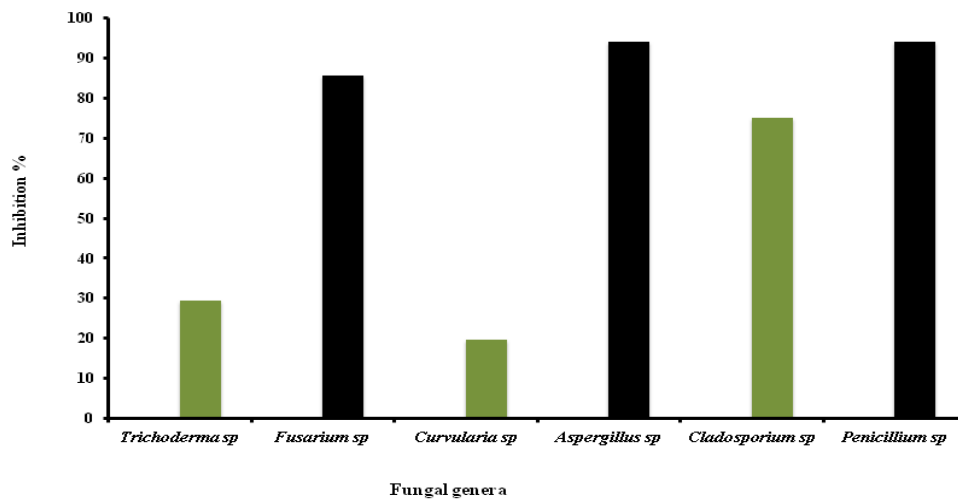


Fig.11 Antifungal activity of *Clerodendrum infortunatum* L. against some dominant fungal genera.

DISCUSSION

The study showed that fungal spores were present throughout the study period in all the sampling environments. Seventeen genera were identified based on the spores collected by the non-viable methodology. These were *Ascospore*, *Aspergillus* sp, *Penicillium* sp, Basidiospore, *Cladosporium* sp, *Curvularia* sp, *Drechslera* sp, *Fusarium* sp, *Lasiodiplodia* sp, *Leptosphaeria* sp, *Nigrospora* sp, *Periconia* sp, *Pithomyces* sp, *Sporidesmium* sp, *Torula* sp, *Agrocybe* sp and *Trichoderma* sp and other spore types. Similar type of fungal spore reported in many literatures (Kallawich *et al.*, 2015; Hasnain *et al.*, 2012).

In case viable fungi total 12 fungal genera were isolated from both environments, namely *Aspergillus* sp, *Bipolaris* sp, *Aurobasidium* sp, *Penicillium* sp, *Arthroderma* sp, *Cladosporium* sp, *Curvularia* sp, *Trichoderma* sp, *Fusarium* sp, *Alternaria* sp, *Cochliobolus* sp and *Mucor* sp. Occurrence of these fungal spore in the air reported by many researchers (Chakraborty *et al.*, 2001; Adhikari *et al.*, 2004).

Results of this study revealed outdoor fungal spore concentration was higher than indoor. This may be due to in indoor environments, proper air ventilation, cleaning procedure, use of air conditioner; low relative humidity and temperature in indoor environments resulting in the reduction of the abundance of airborne mold spores from outdoor environments. This result showed proximity with the finding (Li *et al.*, 1991; Manzelet *et al.*, 2017).

The result of indoor/outdoor ratio exhibited exogenous source of indoor contaminations. This findings are in agreement with result (Gutarowska *et al.*, 2002; Tsai *et al.*, 2002; Stryjakowska *et al.*, 2007). Kalwasińska *et al.*, (2012) showed similar pattern of occurrence of fungal aerosols in indoor and outdoor air of buildings.

In this present study in the month of March the spore concentration was highest compare to the months its may be due to in this month's temperature, relative humidity and low rainfall favour the growth of the fungus. Premila (2013) reported in the month of March they observed minimum concentration of airborne fungal spores.

Six prevalent non-viable and viable fungal flora were procured from the study sites during the study period. Many researcher throughout the world found that these fungal genera such as *Ascospores*, *Cladosporium* sp, *Aspergillus* sp, *Penicillium* sp and Basidiospores predominating in tropical areas (Hasnain *et al.*, 2013; Ceter *et al.*, 2009; Burton *et al.*, 2010; Quintero *et al.*, 2010).

The antifungal activity of *Clerodendrum infortunatum* L. extract showed highest efficacy against *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp followed by *Cladosporium* sp *Trichoderma* sp and *Curvularia* sp. This finding agreed with result of Kharkwal *et al* (2012) they found *Clerodendrum infortunatum* showed significant inhibitory responses against *Aspergillus* sp and *Penicillium* sp.

As conclusion, aeromycobiota which were detected by Burkard Personal Air sampler and Anderson sampler in the selected environments reported to be highly allergic to human

saprophytic, deteriorating in many literatures. This study provides baseline information on composition of aeromycobiota in those working environments. From observations of antifungal activity of *Clerodendrum infortunatum* it can be suggested extract of the leaves from *Clerodendrum infortunatum* served an effective antifungal agent to reduce the airborne fungal spore concentration. The outcomes of the study along with the insightful explanation could aptly provide basis for strategizing effective preventive measures against airborne-fungi exposure related health risk among the workers working in this environment.

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