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An approach of fungal aerospora in students class-rooms: Identification, biocontrol and its pathogenicity

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According to reports, one of the key elements influencing how people's health is affected in classrooms is the air quality. In the current investigation, pathogenicity of the dominant fungus was evaluated, and the goal was to isolate and identify indoor air fungi dispersed in classrooms.In pure cultures, 11 from 7 genera were isolated and identified. The most isolated fungus, *Trichoderma longibrachiatum* (*T. longibrachiatum),* was eradicated using essential oils (camphor, mint, basil, lavender, rose, and lemon). To determine the pathogenicity of *T. longibrachiatum* on rabbit hematological, biochemical, and histopathological features, oral administration of 50 mg/kg of the organism for two weeks was conducted.*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Cladosporium cladosporioides, Penicillium frequentus*, *Penicillium purporogenum*, *Paecilomyces lilacinus*, *Paecilomyces variotii*, *Rhizopus stolonifer*, *T. longibrachiatum* and *Ulocladium sp*. were isolated from twelve class rooms (CMs). Air drenched with each of camphor and mint oil suppressed *T. longibrachiatum* growth by 100% as compared to the control. While, basil, lavender, rose and lemon oil did not show any antifungal activity. *T. longibrachiatum* oral administration induced a significant decrease in RBCs count, Hb concentration, MCV and MCH values. A remarkable reduction of WBCs count and Neutrophil were observed. Moreover, *T. longibrachiatum* induced a significant elevation of hepatic biomarkers (ALT and AST), No variations were noticed in the values of albumin, LDH activity and Creatinine. Histopathological assay revealed a hepatic and pulmonary deterioration subsequently to *T. longibrachiatum* administration. Taken together, our results showed that *T. longibrachiatum* was the most prevalence isolated fungi from the classrooms indoor air and exerting various biological hazards which requires purifying classrooms indoor air using the natural essential oils.

Keywords: Indoor fungi; essential oil; *Trichoderma longibrachiatum* , pathogenicity

INTRODUCTION

Fungal community estimated by more than 70,000 species, including 350 pathogenic species for human. Fungi are important components of air dust particles (Soleimani et al. 2016). Consequently, their stability and physiological activities as well as the production of spores rises with suitable levels of temperature and humidity especially in closed places that equipped with air conditioning systems (Moustafa and Abdelzaher, 2016). The sick building syndrome, which is characterized by a number of human symptoms (such as dry skin, headaches, and lethargy), was linked to fungi exposure (Cooley et al. 1998 & Singh, 2005). But sick building syndrome has been reported often in schools, hospitals, care facilities, and private residences (Crook and Burton, 2010). To reduce the danger of exposure, fungal contamination must be addressed quickly (Rogawansamy et al. 2015). For proper diagnosis and treatment of concurrent fungal infections, a precise description of the fungus is necessary.

Because *Trichoderma* species are difficult to differentiate morphologically, molecular techniques, such as DNA sequencing and the identification of phylogenetic species compatible with lineages utilizing a number of unwanted genes, are required to provide a reliable identification of *Trichoderma* spp. (Druzhinina et al. 2006). In order to identify the many species of *Trichoderma*, phenotypic traits and genetic studies are frequently combined. Based on morphological characteristics and DNA sequencing data at three loci, the RNA polymerase II subunit (RBP2), translation elongation factor 1- gene (TEF1-), and internal transcription spacer (ITS) of the nuclear ribosomal RNA gene, four new species that belong to the *Harzianum* clade were identified (Innocenti et al. 2019). The non-coding ITS sections of 5.8S RNA are incredibly varied

amongst various species. These conserved sequences can be used to determine how closely related fungi are phylogenetically (Haque et al. 2020).

Indoor places in hot and air-conditioned environments are related to many problems of fungi caused by poor ventilation. Moreover, air fungi are not limited to allergic events and asthma, but also, aspergillosis (Ullmann et al. 2018) as well as liver and kidney tumors in humans and animals (Creppy, 2002). A decrease in the quantity of neutrophil and monocytes in the peripheral and peritoneal cavity after IP administration of *T. asperelloides* spores were reported (Santos et al. 2017). Trichoderma, *Fusarium* and *Stachbortrys* spp produce mycotoxins which could affect multiple organs (lungs, nervous system and musculoskeletal system) (Ratnaseelan et al. 2018).

Natural fumes of some volatile essential oils control a large number of fungal spores and conidia (Nazzaro et al. 2017). It was reported that oil can be considered as natural antagonists of various pathogens at a lower cost and more safety (Durović-Pejčev et al. 2014). Six essential oils were tested for their fungicidal effects against *Trichoderma agressivum*, *F. europaeum*, the main fungus responsible for the green mold illness (Schroder et al. 2017). The highest antifungal action was found in the oils of peppermint (*Mentha piperita*) and basil (*Ocimum basilicum*). *Eucalyptus* and lavender oils also shown modest antifungal activity against fungus isolated from air, but clove oil was determined to be the most effective antifungal agent (Dianez et al. 2018). Limonene and vinegar had only weak antifungal effects against the isolated species. Clove oil, rose geranium, peppermint, and patchouli were found to effectively prevent mushrooms' and vegetables' fungal infections in a related investigation (El-Fadaly et al. 2018). According to the width of the inhibitory zone, clove oil had the strongest antifungal impact, followed by cinnamon oil and basil oil. *Penicillium* sp. had the greatest value of the clove oil inhibition zone, followed by *Aspergillus glaucus, A. niger, A. nidulans*, and *E. nidulans* (Ainsworth, 2001).

The main objective of this study is to isolate and identify indoor air fungi distributed in classrooms, determine whether sequencing analysis of the internal transcribed spacer region (ITS) of ribosomal DNA could be used to reveal the phylogenetic relationships of *Trichoderma* isolates. Moreover, clarifying the potential harmful effects of one of the most isolated taxa on mammalian physiological processes. Furthermore, introducing a trial to reduce the spread of fungal spores and conidia by using volatile essential oils as a source of disinfecting indoor air are also investigated.

MATERIALS AND METHODS

Collection of samples and fungal isolation from the halls and classrooms indoor air:

Air-spores from different classrooms were isolated as following: Petri dishes containing rose-bengal-PDA were prepared for isolation of fungi from air of halls and classrooms. In places designated for isolation of fungi, plates were opened for an hour until the fungal spores and conidia fell on them without any human intervention. Information was written on the plates after closing and the parafilm was attached to its edges, then placed in plastic bags and incubated at a temperature of 26 °C in the dark until the appearance of fungal colonies.

Purification and Identification of fungal colonies:

After fungal colonies appeared without overlapping, inocula were taken from the middle of each colony and transferred to a Petri dish containing PDA without rose-bengal under aseptic conditions. Incubation was undergone at 26 °C, for 7 days, or until the full appearance of fungal colonies.

Keys from (Moubasher, 1993) and Dictionary of the Fungi (Tasca and decarrli, 1999) were used to identify different species. On the basis of ITS rDNA sequence analysis, *T. longibrachiatum* was also recognized using molecular criteria. Utilizing the ITS universal primer, the ITS gene was amplified. The PCR products were then purified using the OmegaTM PCR Purification Kit (Siddiquee et al. 2007). The PCR products were purified before sequencing them, and the sequence of the ITS rRNA gene underwent a BLASTn search to determine the degree of similarity between sequences [Mkumbe et al. 2018]. In addition to downloading the chosen secondary structures for the examined isolate in a Vienna file format from Mfold server [Meyer and Miklos, 2007], the GC% for the recorded sequence was determined using the ACUA program (Umashankar et al. 2007).

The recorded sequence was aligned using Clustal X version 2.0 software (Chitrakani et al. 2019) with 8 related sequences gathered from the Gene bank (Table 1), and the phylogenetic analyses were carried out using MEGA 4.0 (George and Ramteke, 2019) for neighbor-joining and bootstrap analysis. With branch lengths expressed in the same units as the evolutionary distances used to estimate the phylogenetic tree, the phylogenetic tree is presented at scale. The greatest composite likelihood approach was used to calculate the evolutionary distances (Tamura et al. 2011).

Table 1: List of *Trichoderma* species, aligned sequences length, Gene bank accession No. and CG content.

Species	Aligned sequence length (bp)	Accession NO.	CG content
Trichoderma longibrachiatum	506	MW459192	58%
	522	HG964315	57%
	637	LC577103	55%
	596	MT328534	56%
	599	MZ707301	56%
Trichoderma harzianum	600	MW386645	54%
	559	MN096888	49%
Trichoderma viride	554	LC092110	58%
	540	FR717914	55%

Antifungal activity of the volatile essential oils:

Effect of Camphor, Mint, Basil, Lavender, Rose and Lemon volatile oils on mycelium growth and conidial production of *T. longibrachiatum* was carried out as followed:

T. longibrachiatum was cultivated on PDA medium, in the middle of 9 cm diameter petri dish. The dish without its led was placed in the middle of another 15 cm diameter dish supplemented with 5 ml of each type of volatile oils (*were* purchased from the local markets of the Sakaka city*)* and then the large dish was closed and wrapped with parafilm in order to prevent oil vapors from leaving the dish. Dishes were incubated at 26 º C for 7 days in the dark. Control dishes serving for each fungus by adding sterile distilled water instead of oil. After incubation, readings were taken and compared to control samples.

Study the toxicity of *T. longibrachiatum:*

In vitro* Hemolytic activity of isolated *T. longibrachiatum:

The hemolytic activity of the isolated *Trichoderma* was assessed in vitro using fresh human red blood cells according to the method of (Mukherjee and Rajasekaran,2010 & Chen et al. 2014); briefly 900 µl of ($1X10^6$/ml) fungal spores were incubated with 100 µl of washed three times erythrocytes. After 24 hr in 37˚C in the incubator, the mixture was centrifuged (1500 r.p.m /10 min). Absorbance of the supernatants and of treated and controls samples were measured at 540 nm (A540). 0.9%. As minimum and maximum hemolytic controls, saline and distilled water were employed. There were three duplicates of each experiment.

%Hemolytic activity= absorbance of sample – absorbance of saline / absorbance of dis water X 100

Study the toxicological effects of *T. longibrachiatum* on rabbits (*in vivo* study):

This study carried out using 5 rabbits /group. The experimental animal groups were divided as followed: 1st group was orally administered dis water to serve as control group, 2nd group was orally administered with (50 mg/kg) crude *T. longibrachiatum* for 2 weeks. Blood was immediately collected from each rabbit into a heparinized test tube for hematological assay measuring (RBCs, Hb, MCH, MCV and WBCs). Another blood sample were collected in nonheparinized test tube, serum was isolated and immediately stored at - 20°C till used for the biochemical assay (ALT, AST, albumin, LDH, creatinine). Liver, lung and spleen of the control, and treated animals were fixed in formalin (10%) for histopathological assay. Hematoxylin-eosin-stained sections were captured using Calibrated standard digital microscope camera (Tucsen ISH1000 digital microscope camera), Olympus® CX21 microscope and "IS Capture" software for capture and image enhancements.

Statistical analysis:

Unpaired t-test was used for statistical analysis. The standard error of the mean (SEM) is shown as error bars. For statistical analysis, GraphPad Prism software (Version 5.0, GraphPad Software, La Jolla, CA, USA) was utilized. * $P < 0.05$, ** $P < 0.01$.

RESULTS AND DISCUSSION

Identification of the collected indoor fungal aerospora:

Table [2] illustrated those 11 species belonging to 7 genera were isolated from 12 classrooms in collecting places. A. flavus (JU-F 0045), *T. longibrachiatum* (JU-F 0054), A. niger (JU-F 0046) were steadily the most frequent fungi, *T. longibrachiatum* (JU-F 0054) was recovered from 12 samples matching 24.9 % of total count fungal isolates while *A. flavus* and *A. niger* was recovered from 11 samples matching 14 % of total count [Fig. 1].

Table 2: Total counts of fungi recovered from 12 air samples from classrooms on PDA agar at 27°C.

Species Name	Isolation remarks		
	number of isolates	occurrence remarks	Percentage of total counts
***A. flavus* (JU- F 0045)**	77	11H	15.7
***A. niger* (JU- F 0046)**	77	11H	15.7
***A. terreus* (JU- F 0047)**	22	7M	4.5
***C. cladosporioides* (JU- F 0048)**	39	9H	7.9
***P. frequentus* (JU- F 0049)**	33	10H	6.8
***P. purporogenum* (JU- F 0050)**	22	7M	4.5
***P. lilacinus* (JU- F 0051)**	22	6M	4.5
***P. variotii* (JU- F 0052)**	33	10H	6.8
***R. stolonifer* (JU- F 0053)**	17	3L	3.5
***T. longibrachiatum* (JU- F 0054)**	137	12H	27.9
***U. spp* (JU- F 0056)**	11	2R	2.2
Number of genera	7		
Number of species	11		

Occurrence remarks; H = 60% -100.0%, M = 33 - 59.0%, L = 20 - 32%, and R = 7 – 19%.

Figure 1: Total fungal colonies from classrooms cultured on PDA media after 7 days at 27°C, in the dark.

Molecular identification:

Sequence of complete nuclear ITS rRNA gene was obtained for *T. longibrachiatum* from the PCR product (Figure, 2) and submitted to the GenBank with accession number MW459192. For the isolated species, the CG content was obtained as well as the distinct RNA secondary structure stems and folds was drawn (Figure, 3 A & B).

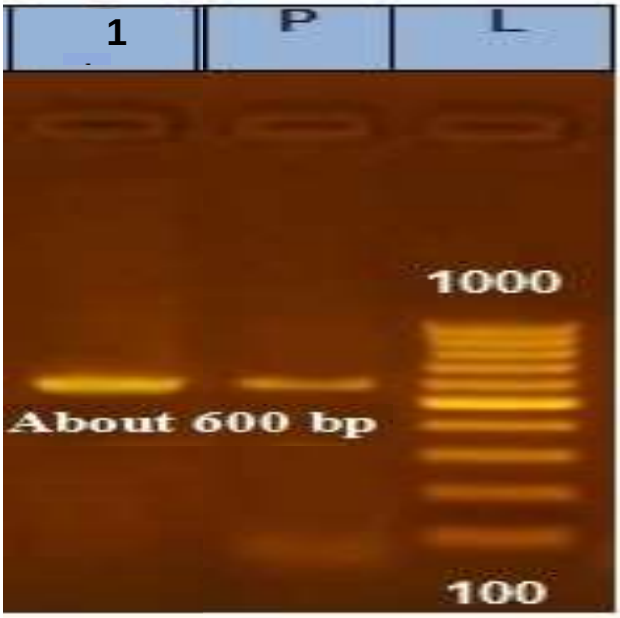

Figure 2: PCR amplification of ITS regions from genomic DNA of *T. longibrachiatum*. A 600 bp DNA fragment amplified using ITS1 and ITS4 primer pair, L= DNA marker (1 kb), P= Positive control, Lanes 1 represent PCR products of *T. longibrachiatum*.

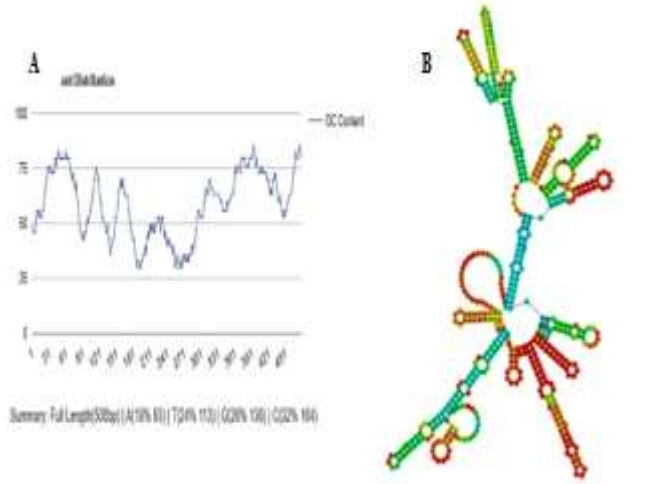

Figure 3: ITS gene sequence analysis of *Trichoderma longibrachiatum* (MW459192). A) Total GC content, B) rRNA Secondary structure.

Phylogenetic analysis was used for the sequence data of nuclear ITS rDNA. The sequence was aligned with 8 highly similar sequences from Gene Bank using the MUSCLE Multiple Sequence Alignment Program (ClustalX version 2.0 software) for neighbor-joining analysis.

The results of Neighbor-joining phylogenetic tree based on the alignment of ITS rRNA gene sequence of isolated species represented that, a closely related species of section *longibrachiatum* (*T. longibrachiatum*) are monophylogenetic (Figure, 4).

Generally, the species related to the same section were monophylogenetic, while the species from different sections were found to be polyphylogenetic. Incidence and prevalence of fungi isolated from of the indoor air on PDA medium at 27°C. was displayed in [Table. 3]

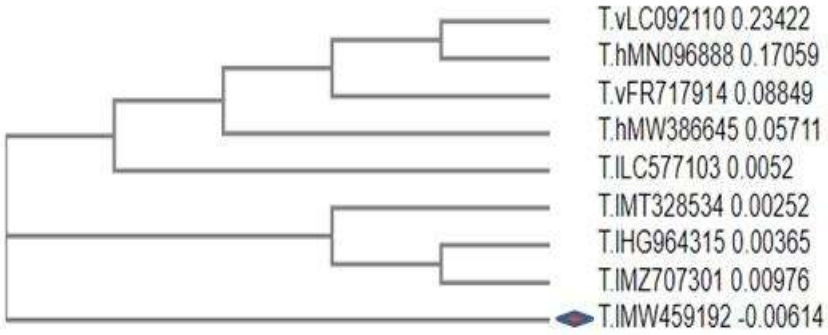

Figure 4: Phylogenetic analysis of ITS region derived from Neighbor- Joining method using MEGA4.0 software. (Diamond shapes represent our isolate and remaining are reference sequences).

Table 3: Incidence and prevalence of fungi isolated from of the indoor air on PDA medium at 27°C.

No. of class room	Fungal species isolated from the studied class rooms			
	No.	%	Fungal Species	
Class room 1	8	72.7	*A. flavus* *A. niger* *A. terreus* *P. variotii*	*C. cladosporioides* *P. frequentus* *P. purporogenum* *T. longibrachiatum*
Class room 2	6	54.5	*A. flavus* *A. niger* *P. variotii*	*C. cladosporioides* *P. frequentus* *T. longibrachiatum*
Class room 3	6	54.5	*A. flavus* *A. niger* *P. variotii*	*P. frequentus* *P. purporogenum* *T. longibrachiatum*
Class room 4	9	81.8	*A. flavus* *A. niger* *A. terreus* *P. variotii* *R. stolonifer*	*C. cladosporioides* *P. frequentus* *P. lilacinus* *T. longibrachiatum*
Class room 5	6	54.5	*A. flavus* *A. niger* *P. variotii*	*C. cladosporioides* *P. frequentus* *T. longibrachiatum*
Class room 6	7	63.6	*A. flavus* *A. niger* *P. variotii*	*P. frequentus* *P. purporogenum* *P. lilacinus* *T. longibrachiatum*
Class room 7	10	90.9	*A. flavus* *A. niger* *A. terreus* *P. variotii* *R. stolonifer*	*C. cladosporioides* *P. frequentus* *P. purporogenum* *Paecilomyces lilacinus* *T. longibrachiatum*
Class room 8	7	63.6	*A. flavus* *A. terreus* *R. stolonifer*	*C. cladosporioides* *P. purporogenum* *P. lilacinus* *T. longibrachiatum*
Class room 9	8	72.7	*A. flavus* *A. niger* *A. terreus* *P. variotii*	*C. cladosporioides* *P. frequentus* *P. lilacinus* *T. longibrachiatum*
Class room 10	7	63.6	*A. flavus* *A. niger* *A. terreus* *P. variotii*	*P. frequentus* *T. longibrachiatum* *P. purporogenum*
Class room 11	8	72.7	*A. flavus* *A. niger* *A. terreus* *P. variotii*	*C. cladosporioides* *P. purporogenum* *P. lilacinus* *T. longibrachiatum*
Class room 12	5	45.4	*A. flavus* *A. niger*	*C. cladosporioides* *P. frequentus* *T. longibrachiatum*

Effect of volatile oils (camphor, mint, basil, lavender, rose and lemon) on the growth of *T. longibrachiatum*:

Current results revealed that air drenched with each of camphor and mint oil suppressed the growth of *T. longibrachiatum* by 100% as compared to the control [Fig. 5]. On the other hand, air drenched with basil, lavender, rose and lemon oil have not showed any effect on growth and sporulation of *T. longibrachiatum*.

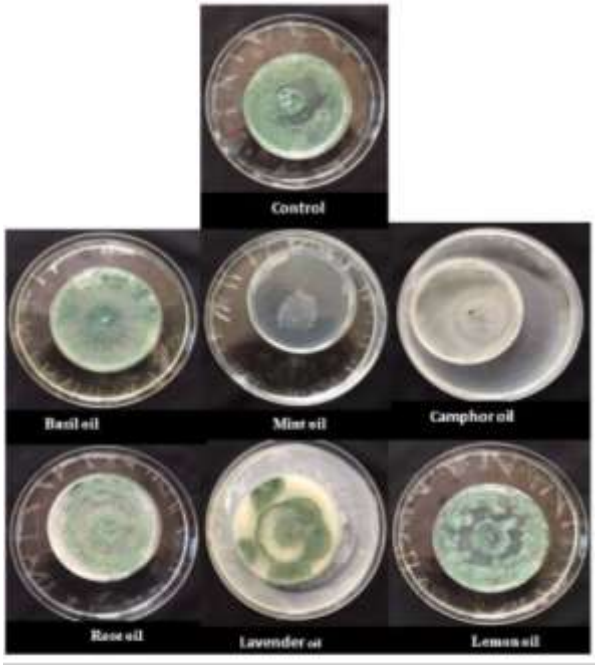

Figure 5: The antifungal activity of volatile vegetable oils [camphor, pepper mint, basil, rose, lavender, lemon] on the mycelium growth and production of *T. longibrachiatum* conidia, after 7 days at 27°C, in the dark.

T. longibrachiatum was extensively present in the classroom air. This fungus is known for its strong role in the biological control to many fungal plant diseases. That taxa aroused our interest, so it was chosen to investigate its growth inhibition naturally and its harmful effect on the experimental animals.

Avoiding the fungal spores and conidia spread in indoor air is a matter of a great concern. In the current study, Camphor and mint oil were proved to suppress the growth of *T. longibrachiatum* by 100%. The antifungal activity of camphor and mint oil could be due to its active ingredients. Camphor (40.54%), linalool (22.92%), cineole (11.26%), and 3,7,11-trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate (4.50%) are the primary components of *Cinnamomum camphora* leaves essential oil [Chen et al. 2014]. Additionally, *Mentha piperita* L. essential oils shown notable antibacterial and antifungal action against gram positive and gram-negative bacteria, as well as yeast and fungus (Reddy et al. 2017). This is mostly due to the primary chemical component's menthol and menthone. Our results are consistent with many previous studies supporting this regard (Moustafa, 2018).

Pathogenicity of T. longibrachiatum:

In vitro hemolytic activity of T. longibrachiatum:

Incubation of human erythrocytes for 24h in ($1X10^6$/ml) *T. longibrachiatum* conidia increased the hemolytic activity with the percentage of 61±4.08 compared to control specimen.

Even though fungal bio-control has numerous benefits. The increased exposure to fungi or fungal molecules may have an adverse effect on human health (Hansen et al. 2010); These adverse effects were confirmed by the current results where *T. longibrachiatum* induced a hemolytic activity after 24 h of incubation human R.B.Cs; the hemolytic activity could be due to peptaibol alamethicin one of *Trichoderma* metabolites which had been shown in previous reports to lyze human erythrocytes, induce metabolic activity in endothelial bovine aorta cells, and release catecholamines from feline adrenal glands [Chung, and Wallace, 2001]. Miscellaneous mechanisms have been shown to be involved in *Trichoderma* sp. antagonism against pathogenic plant fungi (Ruegger et al. 1976, Vinale et al. 2008). *Trichoderma* sp. are the major producers of multifunctional peptides known as peptaibols peptides which have the capability to induce membrane channels in the target organisms (Ruegger et al. 1976, Mikkola et al. 2012). Peptaibol peptides are substances in *T. harzianum* strain ES39 extract are responsible for inhibiting boar sperm motility (Peltola et al. 2004).

It has been shown that the peptaibols are responsible for all of the toxic properties displayed towards the boar spermatozoa through reduction of NADH2 's boar sperm cells, damaging cell membrane of the exposed boar sperm cells. Besides its ability to degenerate mitochondrial membrane potential of human lung epithelial carcinoma cells (Peltola et al. 2004), Peptaibols induce pore forming in the cell membrane which leads to leakage and cell death.

***In vivo* biological effects of *T. longibrachiatum*:**

Hematological and biochemical assays:

Table (4) showed the variation of hematological and biochemical biomarkers induced by oral administration of

50 mg/kg crude suspension of *T. longibrachiatum* for 14 days. A significant decrease in R.B.Cs count and remarkable decline in Hb concentration were reported in *T. longibrachiatum* treated group compared to control group (P ≤0.05, P ≤0.001) respectively. Consequently, blood indices MCV, and MCH were significantly declined. The hemolytic activity could be correlated to the observed decline of RBCs count and subsequently a decrease in Hb content resulting from *T. longibrachiatum* exposure.

Table 4: Effect of oral administration of *T. longibrachiatum* on the hematological parameters.

	Control	T.longibrachiatum
R.B. Cs (10e6/uL)	5.7±0.40	4.3±0.09*
HB (g/dl)	12.04±0.44	9.4±0.31**
MCV (fl)	73.20±2.05	60.50±1.98**
MCH (pg)	32.8±2.9	21.3±0.30*
W.B.C.s (10e3/µL)	3.97± 0.28	2.48±0.59*
Neutrophil %	59.6±7.46	18.8±3.6**

Results expressed as Mean± SE (n=4), *Significantly different comparing with control group. *(p<0.05), **(p<0.01), unpaired t-test.

On the other hand, *T. longibrachiatum* induced a severe leukocytopenia as a reduction in W.B.Cs (accompanied by sever Neutropenia (≤0.01) were reported compared to control group.

The immune-modulatory impact of *Trichoderma* sp. is the subject of intense dispute. By negatively modulating immune system homeostasis, biocontrol agents can impede it. The current findings showed that oral administration of *T. longibrachiatum* has an adverse effect on the innate immune system by reducing total leucocytes and, more particularly, neutrophils. This impact is consistent with (Santos et al. 2017), who demonstrated that IP injection of *T. asperelloides* spores decreased the phagocytic ability of phagocytes exposed to fungal spores as well as the number of neutrophils and monocytes in the peritoneal cavity and peripheral circulation.

Due to the link between low neutrophil counts and a higher risk of infection, they are regarded as the first line of defense against pathogens from outside the body or tissue lesions. The reduction of IL-10 and IFN levels in mouse intranasal *T. stromaticum* spores in broncho-alveolar lavage fluid and splenocyte cultures was also demonstrated (Alves-Filho et al. 2011).

Cylindrocapon lucidum, *Trichoderma polysporum, Fusarium oxysporum*, and *T. harzianun* are biocontrol fungi that generate the immunomodulatory compound known as cyclosporin A (CsA). These fungi have previously been recognized as modulators of the mammalian immune response [Rodríguez et al. 2006, Azam et al. 2012]. The nuclear factor of activated *T lymphocytes* has decreased activity, and CsA is linked to decreased lymphoid cell activation, proliferation, and survival (Rovira et al. 2000).

On the other hand, L-lysine-alpha-oxidase isolated from *T. harzianum* Rifai affect the functional activity of T-lymphocytes. It was shown that parental administration of L-lysine-alpha-oxidase had no suppressive effect on the T-lymphocyte functional activity. Inhibition of macrophages functional activity by L-lysine-a-oxidase was observed. L-Lyzine-alpha-oxidase had a selective lymphotropic action and showed no mytostatic activity, which is an advantage of the enzyme compared to other antitumor agents (Podboronov et al. 2011).

Moreover, Hepatotoxicity induced by *T. longibrachiatum* was represented by a significant elevation of the hepatic ALT and AST activity (P ≤0.05). While, a non-significant variation in albumin, LDH and creatinine values were noticed [Table 5].

Table 5: Effect of oral administration of 50mg/kg *T. longibrachiatum* on the hepatic and renal biomarkers.

	Control	*T.longibrachiatum*
ALT (U/L)	44.20±5.05	68.73 ±6.8*
AST (U/L)	27.70±10.03	90.38±19.81*
Albumin (g/dl)	4.200±0.59	4.650±0.104
LDH (U/L)	174.3±39.60	196.3±60.70
Creatinine(mg/dl)	0.80 ±0.066	0.60±0.057

Results expressed as Mean± SE (n= 4), * Significantly different comparing with control group, * (p<0.05), unpaired t-test

Histopathological Assay:

Oral administration of (50 mg/kg) crude *T. longibrachiatum* for 14 days caused various morphological alterations in liver and lung compared to control group. Small pale-yellow nodules appear in liver as well as lung congestion [Fig. 6].

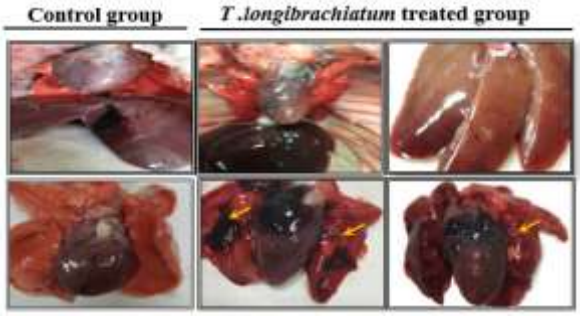

Figure 6: Morphological examination of rabbit's liver and lung of control and 50 mg/kg *T. longibrachiatum* treated groups

Furthermore, microscopic examination of tissue sections revealed that *T. longibrachiatum* caused pathological changes in hepatic tissues represented as marked hydropic degeneration where, with vacuolated cytoplasm and markedly congested central vein, mild peri-portal inflammation (Fig 7 A, B) compared with normal hepatic tissue. Furthermore, *T. longibrachiatum*

infection affect animal lung causing slightly distorted architecture, thickening of the interstitial wall, capillary congestion with mild infiltrate of inflammatory cells and moderate emphysema (Fig 7 C, D). Spleen structure showed marked congestion and expansion of red pulp with decreased size of white pulp lymphoid follicles in *T. longibrachiatum* treated group compared to control sections (Fig 7 E, F).

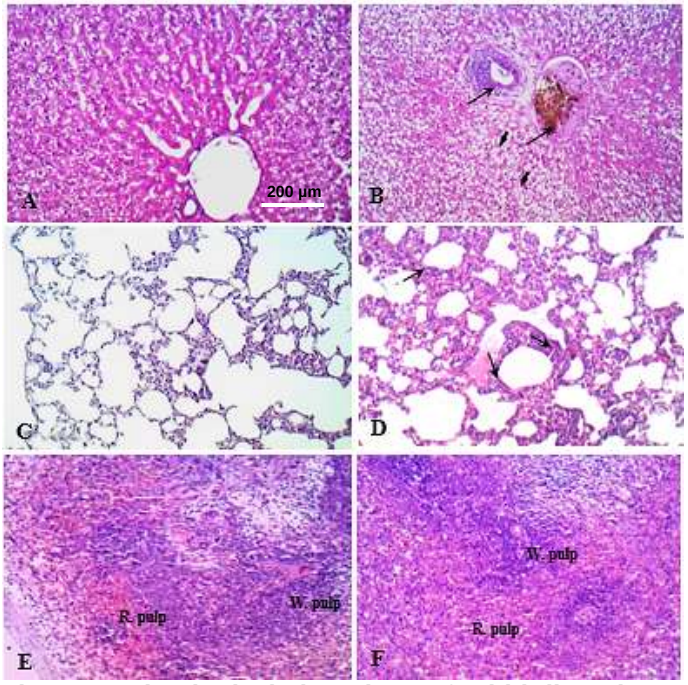

Figure 7: Histological alterations of rabbit liver, lung and spleen after oral administration of 50mg/kg crude suspension of *T. longibrachiatum.* A- Normal hepatic tissue, B-marked hydropic degeneration, congested central vein and mild peri-portal inflammation. C- Normal lung structure, D- thickening of the interstitial wall, capillary congestion with mild infiltrate of inflammatory cells. and E- spleen of control group, F- spleen congestion and expansion of red pulp with decreased size of white pulp lymphoid follicles of *T. longibrachiatum treated group*. Sections were observed at (100X).

Hepatic injury, lung and spleen structure damage were observed in *T. longibrachiatum* administered group. Appearance of these effects consistent with (Guarro et al. 1999) who reported in his necropsy study that the brain showed edema, hemorrhaging with tele-encephalic ventricular dilation; these mycotic lesions were ultimately the cause of the brain hemorrhage. Also, it was observed that the lungs had several microabscesses and there were also some patchy areas around the abscesses of consolidation and acute bronchiolitis. Furthermore, the liver was full of tiny yellow nodules. Organ injury such as sinusitis and pulmonary lesions caused by *T. longibrachiatum* and *T. harzianum* were noticed in intestine, liver and bone marrow transplant recipients especially in neutropenic patients (Furukawa et al. 1998& Guarro et al. 1999 & Richter et al.1999 & Podboronov et al. 2011). Five isoenzymatic activities (beta-1,3-glucanase, beta-1,6-glucanase, cellulase, chitinase, protease) were recognized within 17 biocontrol strains of *Trichoderma* (Kottom et al. 2015). 1,6 glucans are present in the *P. carinii* cell wall contribute to lung cell inflammatory activation during infection (Sanz et al. 2004). Furthermore, spleen of *T. longibrachiatum* treated group characterized by a decreased size of white pulp lymphoid follicles, indicated that it has an immunosuppressive effect.

CONCLUSIONS

In conclusion, A. flavus, A. niger, A. terreus, Cladosporium cladosporioides, Penicillium frequentus, Penicillium purporogenum, Paecilomyces lilacinus, Paecilomyces variotii, Rhizopus stolonifer, Trichoderma longibrachiatum, and Ulocladium sp. were isolated from classrooms indoor air. T. longibrachiatum was the most prevalence fungi were greatly inhibited by camphor and mint volatile oil. T. longibrachiatum induced deleterious effects on the mammalian biological systems. It is worth mentioning that precautions must be taken towards exposing people involved in growing crop plants to this fungus, since that the Trichoderma sp. is used extensively in the biological control of some fungal plant diseases.

Supplementary materials

Not applicable

Author contributions

All authors designed, performed the experiments, analyse the data and also wrote the manuscript. All authors reviewed the manuscript, read and approved the final version.

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All of the data is included in the article/Supplementary Material.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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