Aspergillosis Of Onion, A Concern For Human Health

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***Abstract*—Fungal invasive diseases in human is as important as bacterial, viral and vector borne diseases which causes around 1.3 million mortalities per year. The most common among the fungal diseases is Aspergillosis caused by few species of Aspergillus viz. A.fumigatus, A.flavus, A.terrus and lesser known A.niger. Although, Aspergillus niger is a plant invasive fungal pathogen, it is reported as an unusual cause of invasive pulmonary aspergillosis, tracheobronchitis, fatal pulmonary oxalosis, ear infection and asthama related problems. 2.5% adults who have asthama also have allergic bronchopulmonary aspergillosis(ABPA), which is approximately 4.8 million people worldwide, of which 0.4 million have chronic pulmonary aspergillosis(CPA). Another 1.2 million people are estimated to have CPA after having tuberculosis and over 70 thousand people are estimated to have CPA as a complication of Sarcoidosis. People with weakened immune system of lung diseases are at high risk of developing health problem due to aspergillosis. The number of hospitalization related to invasive aspergillosis in developed country like USA increased at an average of 3% per year at an estimated cost of US dollar 1.2 billion. In a broad US healthcare network of ICU autopsy, aspergillosis was one of the top four most common diagnoses that leads to death.**

The spore load of *Aspergillus* is an important factor in the invasion and cases of aspergillosis. Spore load of less than 5cfu/M^3 area is reported to be safe, whereas more spore load than this is alarming as per international standard for aspergillosis infection.

***Aspergillus niger* has its habitat on farm produce, farm crop residues, decomposing material, vegetables and fruits and thereby in the environment vis-à-vis in hospital ward air; but quite often the spore threshold is not enough for alarming situation. A severe spore load of *A. niger* was found in invasion of onions having onion aspergillosis. When such onions are handled during the trading, in market and by housewives in the kitchen, the *A.niger* spores get released, which were trapped in the air passage in nostril in our experimentation. Such aspergillosis onion, therefore, is a cause of concern for human health in near future and should be taken care of. The present paper insights on different aspects of human aspergillosis and the role of onion aspergillosis as a new source of inoculum for human Aspergillosis.**

Keywords—Onion Aspergillosis, Aspergillus niger, human Aspergillosis, Infection, Human health.

Introduction:

Aspergilloosis is an infection caused by *Aspergillus*, a common mould present everywhere. Most people breath *Aspergillus* spores available in the air without getting risk, may be due to lees spore concentration in the air. However, people with weakened immune system of lung diseases are at a high risk of developing health problems due to *Aspergillus*. The types of healthy problems caused by *Aspergillus* includes allergic reaction, lung infection and infection of other organs.

Infections due to *Aspergillus* species result in significant morbidity and mortality. Most infections are attributed to *Aspergillus fumigatus* , followed by *Aspergillus flavus* and *Aspergillus terreus. A. niger* is less commonly reported as a cause of invasive disease. However, *A. niger* has been associated with otomycosis [1], cutaneous infections [2] and pulmonary disease. There are reports of *A. niger* causing pneumonia and pulmonary infection fatal for a patient who had been on long-term steroid treatment for COPD [3], or had a history of asbestos exposure and tuberculosis [4] or had a history of *Mycobacterium avium* complex causing cavitary disease [5]. All these patients had evidence of heavy calcium oxalate deposition on pathological examination, a sign of *A.niger* as causative agent. A review of COPD patients with invasive pulmonary aspergillosis (IPA) found 3.6% of cases due to *A. niger [6]*. In a case series of eight patients with invasive *A.*

niger infection and haematological malignancies, three were on high-dose steroids, and seven were neutropenic. There was a 75% mortality rate attributed to *A. niger* [7].

Aspergillus niger is mainly a soil and air inhabitant causing Aspergillosis or black mould infection of bulb crops, vegetables and fruit crops. However, their infection in these agricultural commodities does not alarm due to less incidences [8] and is commonly seen as a pathogen of post-harvest diseases. It is also found in indoor environments and as a contaminant of food. The fungus spread is mostly through air where the spore load of *Asergillus* fungus is not a cause of concern for Aspergillosis disease expect in persons having weakened immune system. The availability of higher spore load of *A.niger* in the environment may increase the risk of human Aspergillosis as clinical cases. *Aspergillus niger* as unusual cause of invasive pulmonary aspergillosis [9], tracheobronchitis and invasive pulmonary aspergillosis in lung transplant recipients [10] and pneumonia with fatal pulmonary oxalosis [11] are some of the examples of *A. niger* as aspergillosis pathogen. The present paper insights on the present status of *A.niger*, the aspergillosis it causes, and onion aspergillosis as a new source of *A.niger* inoculum for human Aspergillosis

A Case Study

Present Status of *A.niger* Aspergillois, Distribution of *A.niger* around the world, Onion Aspergillosis as concern and Future Implications:

1. Discovery of *Aspergillus* as a Human Pathogen

A fascinating story of discovery of *Aspergillus* as human pathogen is given by Janet Lee [12] . In the midst of the 1789 French Revolution, Jacques Thibault, a 22 years old soldier experienced severe facial pain with the elevation of his cheekbone and the protrusion of his right eye. When physicians perforated Thibault's alveolar process, a fungal mass was found in the maxillary sinus. Resection could not be performed due to excessive bleeding. Twenty-two days later, the fungal mass had grown and filled part of his mouth and his entire right nostril, causing difficulty in swallowing and breathing. Thibault was admitted to a Paris Hospital where the fungal mass was excised by a surgeon and white heat cautery was applied to destroy any remnants of the fungus. Small fungal growths reappeared three to four weeks later at the back of the sinus and the soft palate; all were cauterized using a branding iron. Mr. Thibault left the hospital 134 days after admission with no subsequent recurrences. Thibault's case was the first recorded infection attributed to *Aspergillus*.

In the years following Thibault's case, other case reports implicated *Aspergillus*. The earliest description of pulmonary aspergillosis was published in 1842 by physician, John H. Bennett [13]. Bennett noted the presence of a fungus in the lungs of a post mortem patient with pneumothorax. The name *Aspergillus* was given to the fungus by Pier Antonio Micheli, an Italian priest and biologist in 1729 when cataloging moulds. The moulds resembled an aspergillum (fig.1.), a holy water sprinkler (from Latin Spargere-to sprinkle).

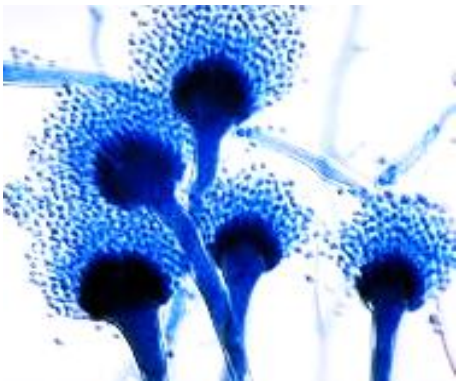

Fig.1. The fungal structure resembling aspergillum

In 1863 the *Aspergillus* species *fumigatus* was first isolated from human lung infections and described by physician Georg W. Fresenius [14]. He observed that the spores had a green pigmentation and had no septated fertile hyphae or conidiophores. *Fumigatus* is derived from Latin "fumigave" which means smoky referring to the smoky blue-gray mycelium.

Subsequent cases of Aspergillus infections over the next several years showed the most frequent species of Aspergillus responsible for human infection was *A. fumigatus.* From 1920 to 1965, cases of disseminated aspergillus infections were implicated in the heart and CNS in addition to the sinuses and lungs.

2. *Aspergillus* species responsible for Aspergillosis in human

There are approximately 180 species of *Aspergillus*, but fewer than 40 of them are known to cause infections in humans. *Aspergillus fumigatus* is the most common cause of human *Aspergillus* infections. Other common species include *A. flavus*, *A. terreus*, *A. niger*, and *A.nidulans* [15] with *A. fumigatus* being responsible for more than 90% of infections followed by by *A. flavus* and *A.niger* [16].

Thus, *Aspergillus fumigatus* is one of the most ubiquitous species responsible for Aspergillosis. Humans and animals constantly inhale numerous conidia of this fungus, present in the environment. The conidia are normally eliminated in the immune-competent host by innate immune mechanisms, and aspergilloma and allergic bronchopulmonary aspergillosis, an uncommon clinical syndromes, are the only infections observed in such hosts. Thus, *A. fumigatus* was considered for years to be a weak pathogen. With increases in the number of immune-suppressed patients, however, there has been a dramatic increase in severe and usually fatal invasive aspergillosis, now the most common mould infection worldwide [17].

3. Types of human aspergillosis

The different types of Aspergillosis can cause different symptoms [18].

3. 1. Allergic bronchopulmonary aspergillosis (ABPA): Occurs when *Aspergillus* causes inflammation in the lungs and allergy symptoms, but doesn't cause an infection [19]. The symptoms of **ABPA** are similar to asthma symptoms, including Wheezing, Shortness of breath, Cough, and Fever (in rare cases).

3. 2. Allergic *Aspergillus* sinusitis: Occurs when *Aspergillus* causes inflammation in the sinuses and symptoms of a sinus infection, but doesn't cause an infection [20]. Symptoms of **allergic** *Aspergillus* **sinusitis** include Stuffiness, Runny nose, Headache, and Reduced ability to smell [21].

3. 3. Aspergilloma: Occurs when a ball of *Aspergillus* grows in the lungs or sinuses, but usually does not spread to other parts of the body [22]. Symptoms of an **aspergilloma** ("fungus ball") include Cough, Coughing up blood, and Shortness of breath.

3. 4. Chronic pulmonary aspergillosis: Occurs when *Aspergillus* infection causes cavities in the lungs, and can be for a long-term (3 months or more). One or more fungal balls (aspergillomas) may also be present in the lungs [23]. Symptoms of **chronic pulmonary aspergillosis** include weight loss, Cough, Coughing up blood, Fatigue, and Shortness of breath [24].

3. 5. Invasive aspergillosis: Occurs when *Aspergillus* causes a serious infection, and usually affects people who have weakened immune systems, such as people who have had an organ transplant or a stem cell transplant or are already sick from other medical conditions. Invasive aspergillosis most commonly affects the lungs, but it can also spread to other parts of the body. Symptoms of **Invasive aspergillosis [18]** includes Fever, Chest pain, Cough, Coughing up blood, and Shortness of breath; other symptoms can develop if the infection spreads from the lungs to other parts of the body.

3.6. Cutaneous (skin) aspergillosis: Occurs when *Aspergillus* enters the body through a break in the skin (for example, after surgery or a burn wound) and causes infection, usually in people who have weakened immune systems. Cutaneous aspergillosis can also occur if invasive aspergillosis spreads to the skin from somewhere else in the body, such as the lungs [25].

Aspergillus niger is commonly regarded as a pathogenic allergen generally associated with lung infections in individuals with weak immune system. Because the conidia and conidiophores are small, readily air borne, can easily breathed in and cause deep or systemic mycosis [26]. Similarly, Ear is the location of *A. niger* infection. Local lesions in both external and middle ear, as well as in post-operative cavities, can create favourable conditions for fungal growth and subsequent otomysis [27], [28]. *A. niger* produce secondary metabolites like oxalic acids, kojic acids abundantly and cyclic pentapeptides having moderate to high **acute toxicity** [29]. Oxalate crystals of oxalic acids produced by *A. niger* can cause pulmonary oxalosis [11].

4. Infection of *Aspergillus niger* causing Invasive Aspergillosis

Invasive aspergillosis caused by *Aspergillus niger* in a chest computed tomography scan (CT) revealed heterogeneous consolidation in the right upper lobe with cavitation (Fig. 2). A sputum culture showed oropharyngeal flora and an unidentified mould which grew in to a culture of *A. niger*. Bronchoscopy found diffusely erythematous and friable mucosa, blood and debris present in the right upper lobe of the lungs

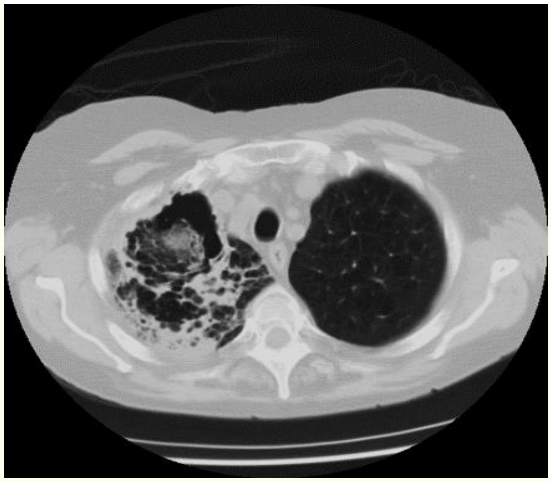

Fig. 2. Chest CT scan of right upper lobe of the lungs with cavitation infected by Invasive aspergillosis. (Source: A.K.Person et.al.2010.J.Med Microbiol.)

The patient with probable invasive *Aspergillus* infection was treated with 200 mg voriconazole orally twice daily. Follow-up in the Infectious Disease Clinic (Duke University Medical Center) and a repeat CT scan 4 weeks later with Bronchoscopy revealed acute and organizing pneumonia, stains consistent with fungal hyphae and oxalate crystallosis (Fig. 3), with repeat cultures persistently growing *A. niger*. Pathology thus fulfilling the criteria for the diagnosis of proven invasive *A. niger* infection [30].

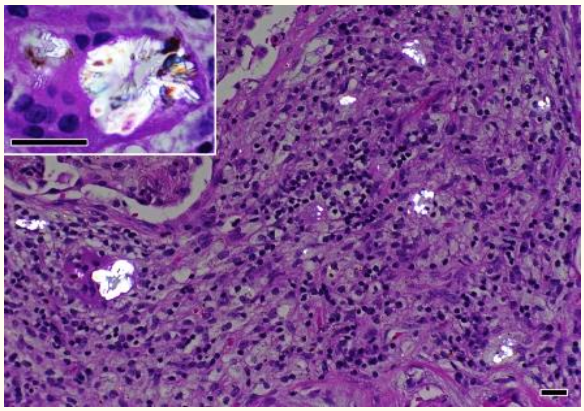

Fig. 3. Surgical pathology of specimens from right upper lobectomy of the lung, showing oxalate crystallosis consistent with *A. niger* infection. Bars, 25 µm. (Source: A.K.Person et.al. 2010. J.Med.Microbiology)

5. Distribution of human Aspergillosis in world

Milder, allergic forms of aspergillosis are more common than the invasive form of the infection.

Allergic bronchopulmonary aspergillosis (ABPA) likely affects between 1 and 15% of cystic fibrosis patients [31].

One study calculated that 2.5% of adults who have asthma also have ABPA, which is approximately 4.8 million people worldwide [32]. Of these 4.8 million people who have ABPA, an estimated 400,000 also have chronic pulmonary aspergillosis [32].

Another 1.2 million people are estimated to have Chronic pulmonary Aspergillosis (CPA) after having tuberculosis [33], and over 70,000 people are estimated to have CPA as a complication of sarcoidosis [34].

Invasive aspergillosis is uncommon and occurs primarily in immune-compromised people. The first population-based incidence estimates for invasive aspergillosis were obtained from laboratory surveillance conducted in the San Francisco Bay Area during 1992-1993 and suggested a yearly rate of 1 to 2 cases of aspergillosis per 1,00,000 populations [35]. However, the epidemiology of invasive *Aspergillus* infections has likely shifted since this time due to the increasing number of solid organ and stem cell transplant recipients and newer immune-suppressive agents. The number of hospitalizations related to invasive aspergillosis in the United States increased with an average of 3% per year during 2000- 2013 [36]. Nearly 15,000 aspergillosis-associated hospitalizations occurred in the United States in 2014, at an estimated cost of $1.2 billion [37].

Prospective surveillance among transplant recipients performed during 2001-2006 found that invasive aspergillosis was the most common type of fungal infection among stem cell transplant recipients [38] and was the second-most common type of fungal infection among solid organ transplant recipients [39]. In a broad US healthcare network of intensive care unit autopsy studies, aspergillosis was one of the top four most common diagnoses that likely lead to death [40].

5.1. Aspergillosis outbreaks

Although, most cases of aspergillosis are sporadic (not part of an outbreak), outbreaks of invasive aspergillosis occasionally occur in hospitalized patients. Invasive aspergillosis outbreaks are often found to be associated with hospital construction or renovation, which can increase the amount of airborne *Aspergillus*, resulting in respiratory infections or surgical site infections in high-risk patients [41], [42]. Outbreaks of primary cutaneous aspergillosis and central nervous system aspergillosis in association with the use of contaminated medical devices have also been described [43], [44]. The incubation period for aspergillosis is unclear and likely varies depending on the dose (spore load) of *Aspergillus* and the host immune response.

5.2. Deaths due to aspergillosis

Allergic forms of aspergillosis such as allergic bronchopulmonary aspergillosis (ABPA) and allergic *Aspergillus* sinusitis are generally not life-threatening.

In contrast, although invasive aspergillosis is uncommon, it is a serious infection and can be a major cause of mortality in immune-compromised patients. A large prospective study found the one-year survival for people who had invasive aspergillosis and 59% of them were solid organ transplant recipients [40] while 25% of them were stem cell transplant recipients [38]. In a systematic review of intensive care unit autopsy studies, aspergillosis was one of the top four most common diagnoses that likely lead to death [45].

The presence of Aspergillosis causing fungi in the hospital wards were the most common infection sites for the patients. Matos et.al [46] in microbiogical assessment of indoor air quality of different hospital sites reported that fungal spore level varies over time and with other factors, such as ambient temperature and humidity. An exposer level of less than 5 cfu/M^3 of Aspergillus species in protective isolation area with limit of 15 cfu/M^3 for gross colony of all fungal organism is recommended at international level to be safe. The most predominant species of Aspergillus isolated in the hospital air in chandigarh, India, were *A. flavus , A. fumigatus* and *A. niger* [47] in Air conditioned and non-air conditioned wards which varies during different months of the year (table 1). The average spore count in AC and non AC areas was 82 cfu/M^3 and 122cfu/M^3 respectively. In AC areas during the year the range of *A. flavus* was 0 to 40.6, *A. fumigatus* 0 to 40.6 and *A. niger* 0 to 43.7 which was more than the international standard of safe level of Aspergillosis infection.

Table.1. Month-wise average of *Aspergillus* spore in AC and Non-AC areas of hospital at PGI, Chandigarh, India.

Month	***Aspergillus* colony forming unit(cfu)/M3 area for AC/Non-AC areas**		
	A.flavus	***A.fumigatus***	***A.niger***
Jan	23.4 / 45	10.9 / 3.1	3.1 / 16
March	40.6 / 74.9	9.3 / 6.2	43.7 / 5.2
April	2.7 / 32.5	0 / 0	6.2 / 32.5
May	0 / 4.5	22.9 / 63.6	25 / 46.5
June	75 / 45	9.3 / 0	0 / 45
July	21.9 / 41	0 / 2	25 / 45.8
Aug	59.3 / 37.5	40.6 /7.2	0 / 133
Sept	29.6 /32.3	7.8 / 17.7	7.8 / 26.4
Oct	20.3 / 43.7	10.9 / 14.6	3.1 / 59.3
Nov	12.5 / 29.1	12.5 / 0	12.5 / 25
Dec	12.5 / 16.6	25 / 18.7	12.5 / 8.3

(Source: Rudramurthy et.al, 2016. Indian J Midical Microbiology.34(4): 529-532).

Similarly, the spore load of *Aspergillus* species responsible for human aspergillosis in the environmental air of vegetable and fruit market in mumbai (table2) indicate that the spore load and percent contribution of *A.niger* in the spore load was more among all the aspergillus species and was followed by *A.flavus* and *A. fumigatus*. Thus the spore load of Aspergillus niger was observed to be at least 2 to 3 times more than it is reported from the hospital ward areas and normal environment.

Table2. Quantification of important *Aspergillus* species in environmental air of vegetable and Fruit market in Mumbai, India.

Aspergillus	**Spore/M^3**	**Percent contribution in total spore load**
Spore count of important *Aspergillus* species		
Aspergillus candidus	129	2.6
A.flavus	258	5.1
A.fumigatus	126	2.5
A.niger	321	6.4
A.terrus	46	0.9

(Source: Kakde and kakde,2012. Acta Bot.Croat.71(1): 147-157)

6. Aspergillosis of Onion bulbs as source of *Aspergillus niger*.

The fungi causing Onion Aspergillosis or black mould in onion bulb is *Aspergillus niger*. Mild infections of the fungus in onion bulbs is generally not of major concern, however the severe infection in onion bulbs (fig 4) with high Aspergillus spore densities may pose a problem of respiratory tract infection while handling or utilising such severely affected onion in the household.

Fig 4. Aspergillosis infected onions in market

6.1. Global Distribution of Onion Aspergillosis fungus

The fungus *Aspergillus niger* causing Onion Aspergillosis is present in several countries around the world (Fig.5) and may serve as a cause of human aspergillosis.

Fig.5.Distribution of Onion Aspergillosis fungi around the World
(source: Cabi.org/isc/datasheet/7444).

The distribution of Aspergillus niger in onion is worldwide specially in Africa (Algeria, Benin, Botswana, Burkino faso, Cameroon, Cote d'Ivoire, Egypt, Ethiopia, Ghana, Guinea, Kenya, Libya, Malawi, Morocco, Nambia, Niger, Nigeria, Reunion, Senegal, Somalia, South Africa,, Sudan, Uganda, and Zimbabwa), in Asia (Bangladesh, China, India, Indonesia, Iran, Iraq, Isreal, Japan, Malaysia, Myanmar, Nepal, Oman, Pakistan, Phillippines, Saudi Arabia, South Korea, Syria, Taiwan, Tajikstan, Thialand, Turkey, Vietnam, and Yemen), in Europe (Czechia, Yugoslavia, France, Germany, Greece, Italy, Luthuania, Moldova, Poland, Portugal, Romania, Russia, Serbia and Montenegro, Slovakia, Spain, and UK), in North America (Cuba, Mexico, Puerto Rico, USA), in Oceania (Australia, Fiji and New Zealand), and South America(Argentina, Brazil, Chile and Venenzuela). (Cabi.org/isc/datasheet/7444).

Besides onion, the fungus also survives on garlic in New Caledonia; on cassava, citrus, peanut, rice soil in Papua New Guinea; on *Piper* leaves, and rice in Solomon Islands, and on peanut in Vanuatu. Globally seeds, bulbs, tubers, fruits and flowers are habitat of this fungi besides its survival in the soil, living on crop remains and any other decayed plant material. Spread occurs as spores blown in the wind and pollute the environmental air with aspergillosis causing spores.

7. Symptoms of Aspergillosis in onion bulbs

In black mould infected or Aspergillosis onions, the *A. niger* develops between the outer (dead, flaky) skin and the first fleshy scales of the bulb. The black spore mass of the fungus can be seen on or between the outer scale of the onion bulbs, especially along the veins. Sometimes whole the inner scale is covered with the masses of black spores of *A.niger* (Photo 6). If conditions are dry, the outer area of the onion bulb dries and shrivels, revealing black spore masses between outer scales. Secondary invaders are common and transform the bulb into a soft, watery mass if the storage conditions are humid.

Fig 6. Presence of *Aspergillus* fungus beneath the onion scale

Black mould has been reported causing major rots of stored onions in the USA and in Japan when kept at high temperatures (27°C) and more than 70% relative humidity. Heavy losses have also been reported from the Sudan. Heavy losses mean heavy infection of *Aspergillus* fungus meaning severe spore load and inoculum for the air pollution with aspergillus spore to cause human aspergillosis, a fungal infection.

Fungal infection is as much important as other diseases in human. The mortality due to fungal diseases are parallels to malaria and tuberculosis and is estimated to be around 1,350,000 patients per year [48]. A large number of fungi causing human disease are known to enter human body through the respiratory tract and causes diseases in susceptible host both in hospital setting and in community [47].

Though, *A.fumigatus* is more encountered as a cause of human Aspergillosis as compared to *A. niger*, the frequency in invasive infection due to *A.niger* is at 5 %

[49] and should not be ignored it as an important causative agent for Aspergillosis.

8. How Aspergillosis of Onion bulb is a concern for human health

Nagerabi and Ahmed [50] reported the spore load of *Aspergillus niger* in red skin and white skin onions in sudan. The incidence and sporulation of Aspergillosis in red skinned onion cv. Saggai, in the stored onion, was 70.5 to 92.5% with 15.8 to 25.3 x 10^6 spores load per gram of onion tissues, while in white skinned cv. El Hilo the incidence of Aspergillosis was observed on 51.5 to 65.0% onions with the spore loads of 12.6 to 20.6 x 10^6spores g-1 of onion tissues.

This spore load when release in to the surrounding environment in the form of a spore dust and spores (Fig.7) or in the kitchen while cutting and using the onion, may serve as an inoculum to cause Aspergillosis in human.

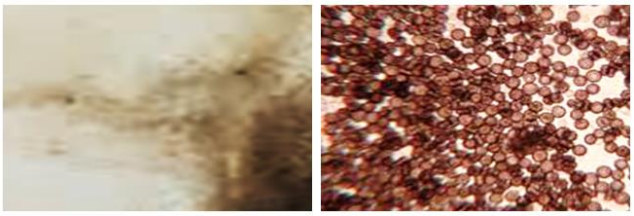

Fig.7. Dust of *Aspergillus* spore masses release from onion bulb and spores of *Aspergillus*

In a simple experimentation, a housewives using the Aspergillosis onion and exposed to the Aspergillosis spore was assessed for the inhalation and presence of Aspergillus spore in the nostril. A sterile moistened swab was used to collect the Aspergillus spore entered in to the nostril (fig. 8) and then plated on the fungal growth medium (potato dextrose agar, composition: potato extract of,250 g., dextrose,20g., agar,20g, distilled water, 1L.,pH,7.0) gave the growth of *A.niger* fungus indicating that during the use of the Aspergillosis onion, the Aspergillus spores can inhale by the housewives or the persons handling these and thus are exposed to the infectious inoculum of Aspergillosis.

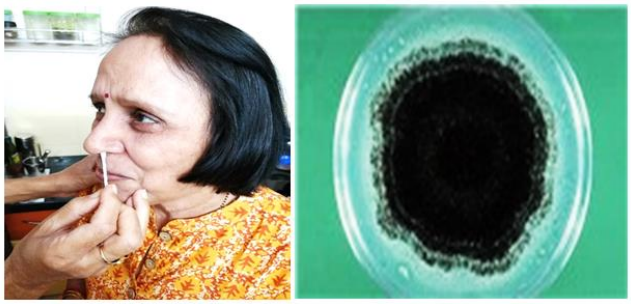

Fig.8. Collection of *Aspergillus* spores from nostril and its growth on Culture media

Discussion

Although Invasive Aspergillosis is a well-recognized clinical entity, invasive disease caused by *A. niger* is less common as compared to *A. fumigatus* and other *Aspergillus* species. The diagnosis can be complicated by the need to distinguish colonization from infection. The potentially aggressive nature of *A. niger*, the utility of calcium oxalosis in histopathological examinations, and the importance of monitoring serum voriconazole concentrations, especially in the setting of progressive disease demonstrate the invasion of *A. niger*.

The diagnostic dilemma in determining of the aetiology of the patient's cavitary lung disease attributed to *A. niger*, can be based on the pathogen recovered from sputum samples. However, it can be difficult to distinguish between colonization and infection when *Aspergillus* is found in the lungs. Presence and detection of *Aspergillus* in the lower respiratory tract was associated with invasive disease in a study of patients with haematological malignancies or those undergoing haematopoietic stem cell transplantation [51]. However, among lung transplant recipients, recovery of *Aspergillus* rarely resulted in progression to overt infection [52]. A review of COPD patients with IPA highlights the need to distinguish between colonization and infection, and suggests a diagnostic algorithm for these patients. Sputum cultures alone may not be helpful because out of 56 COPD patients with IPA, only 12 (21.4%) had cultures positive for *Aspergillus* [6]. Thus, serological tests (such as the galactomannan antigen assay) and radiography tests (with 'halo sign' and 'air crescent sign' on CT being highly suggestive of IPA) must often be combined with microbiological/histological data to establish a diagnosis of true infection. Conclusive culture data or histological evidence of IPA may necessitate bronchoscopy or lung biopsy.

A key feature in the diagnosis of *A. niger* infection is the presence of calcium oxalate crystals on pathological examination. Oxalic acid precipitates and forms crystals when produced via a fermentation process by *A. niger*. The association of calcium oxalate crystals with *A. niger* infection has been repeatedly demonstrated, and it has been suggested that even in the absence of visualized conidia, the presence of these crystals may indicate *A. niger* infection [53]. Both calcium oxalate crystals and numerous conidia can be seen in patient's pathological specimens, pointing to *A. niger* as the aetiological agent of patient's cavitary lung lesions.

Voriconazole is considered the drug of choice for Invasive Aspergillosis [54]. There had been reports of Aspergillosis fungi forming resistance to Azole and these are termed as Azole resistant Aspergillosis, however, in the USA, the resistance of *Aspergillus* to azoles is uncommon [55].

The results of surveillance study on hospital air in AC and non-AC areas shows that there were high frequencies of fungal spores in the hospital air regardless of whether the rooms were AC or not, and further there were seasonal variation in the distribution of the fungi with regards to the average spore counts. Similarly, the spore load of Aspergillus species responsible for

human aspergillosis in the environmental air of vegetable and fruit market indicate that the spore load and percent contribution of *A.niger* in the spore load was more among all the aspergillus species and was followed by *A.flavus* and *A. fumigatus.* Thus the spore load of *Aspergillus niger* was observed to be at least 2 to 3 times more than it is reported from the hospital ward areas and normal environment.

Although there are no strict numerical guidelines for determining the level of fungal contamination in hospital air, the national guidelines of the United Kingdom for prevention of nosocomial aspergillosis for interpretation of the fungal spore burden state that the fungal spores in air should be ≤5 conidia/M^3 in the absence of air filtration. The level more than this limit is considered as high fungal spore burden in hospital's air. Historically, several reports of outbreaks of invasive aspergillosis attributed to construction have been reported in bone marrow transplant, renal transplant, acute leukemia and other immunocompromised patients. However, there is report of non-construction-related invasive aspergillosis also that occurred in patients with chronic obstructive pulmonary disease on mechanical ventilation following air filter change. It has been shown in the past that concentrations of *Aspergillus* spp. below 1 cfu/M^3 may be sufficient to cause infection in high-risk patients. The average spore counts in the AC area and the non-AC area were 82 and 122 cfu/M^3 respectively at a hospital in Minneapolis, USA. Although *A. flavus* is the second most *Aspergilli* to be isolated from human cases worldwide, it is more common than *A. fumigatus* in air in India and certain other countries. However due to less scientific literature available on *A.niger* caused Aspergillosis, the counts of this species is not properly studied though its presence is much more in the air, and on vegetable and fruits available at several places. The aspergillosis infected fruits and vegetables particulary the onions infected with aspergillosis fungi *A.niger* can be a future threat for human Aspergillosis. In our studies the *A.niger* was detected in the nostrils of the persons coming in contact with this fungus. Considering the above fact and that the concentrations of *Aspergillus* spp. below 1 cfu/m^3 may be sufficient to cause infection in high-risk patients and new group of patients without classical risk factor of neutropenia like chronic pulmonary disease, critically ill, and HIV, such high spore concentrations detected in the infected onions may potentially increase the risk for the development of invasive aspergillosis in patients attending hospital and immune-compromised patients.

Conclusion and Recommendation

The Aspergillosis caused by *A.niger* is emerging as a threatening disease for the immune-compromised patients. The spore load of *A. niger* in the environment air is much more than the international prescribed limit. The presence of *Aspergillus* spores is ubiquitous, inhabiting the hey, decomposing material, in field crop residues, on marketed fruits and vegetables. Now the new source of high density *Aspergillus niger* sporulation is found in the form of onion Aspergillosis. These infected onion poses a threat to persons in onion trading and the housewives handling these infected onions in their kitchens. It is therefore recommended that

1. The persons handling the Aspergillosis onions should wear the cloth mask as we are using it for prevention of covid infection, to safeguard themselves from the inhalation of *Aspergillus* spore.

2. In kitchen, the onion should be washed in the running tap water along with the outer skin so as to minimise the spore release in the environment.

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Conflict of Interest:

There is no conflict of interest among the authors, the institute and Hospital

Guidelines for lab animals:

No lab animals were used in the present investigation.

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