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Studies on diversity of seed borne fungal flora of *Vigna unguiculata* (l.) Walp from Nagpur region, MS, India

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ABSTRACT

Vigna unguiculata (L.) Walp is a protein-rich food legume providing seeds for human food, fodder for livestock, soil improvement benefits through nitrogen fixation. The blotter and agar plate technique as recommended by International Seed Testing Association are applied to routine seed health test for detection of seed born fungal pathogens from the seed sample. A population of 21 fungal species fall under 12 genera has been isolated from infested stored seed samples. A count of 10 isolates belonging to 6 genera of diverse fungal group confined on blotter as well as agar plate. Only 6 isolates representing 3 genera restricted to blotter paper while 5 genera each with single species confined only to agar plate as internal seed born contaminants. Ascomycota dominated with highest count of isolates followed by Deuteromycota and Zygomycota. Basidiomycota did not appear on the seed surface. Oomycota had single genera with individual species. Zygomycota represented 3 genera each with individual species. Ascomycota had fungal population of 11 species belonging to 3 genera. Amongst the total 21 isolates, *Aspergillus* dominated with 9 species, exhibiting higher count of species. *Curvularia* is subdominant with 2; while remaining genera had single species. Ascomycota dominated with half of the total incidence followed by Deuteromycota, and fungal incidence. Zygomycota and Oomycota had least incidence. Out of the total 56.3 percent incidence was recorded on blotter paper again 43.6 percent on agar jelly. Among the seed health test techniques, standard blotter paper method was proved comparatively superior over agar plate.

Keywords: *Vigna unguiculata* (L.) Walp, mycoflora; isolate, incidence, infestation

INTRODUCTION

The pathogen free seeds are considered as the vital factor for desired plant population and good economic harvest as seeds constitute the basic input for crop production. During pre- and post-harvest and even in

storage seeds are contaminated with diverse population of fungal organisms. In response to environmental conditions of high relative humidity, optimum temperature and high level of seed moisture content, some of the pathogens attack the seeds in the field internally contaminating the seed testa, embryonic tissues, endosperm and other storage parts. The seeds are carrying disease causing pathogen leading manifolds loss to the crop and reduce the agricultural productivity (Pscheidt & Ocamb, 2014).

Vigna unguiculata L. is a protein-rich indigenous food legume of Africa and Asia where most people used it as main supplement food. Growing in tropical and subtropical regions of the world. It is cultivated mostly cultivated in tropical and subtropical regions of the World for its edible seeds, green pods and leaves which are consumed as vegetable and dry seeds are used in various food preparation. The seeds are reported as an inexpensive source of vegetable proteins containing 24.6% protein, anti-nutritional factors and several vitamins, minerals (fibers, iron, potassium) various nutrients, amino acid amino acid tryptophan, lysine and low fat (Wikipedia, 2020). Besides, it provides fodder for livestock, soil improvement benefits through nitrogen fixation, forage, hay, green manure and cover crop which maintain the productivity of soils (D'Souza, 2019). It is tolerance of shading and can be combined with tall cereal, Sorghum and Maize. Being a drought-tolerant and warm weather crop, it is a promising food and forage species in a typical tropical lowland climate (D'Souza, 2019).

Global cowpea production grew at an average rate of 5%, with 3.5% annual growth in area and 1.5% growth in yield, and the area expansion accounting for 70% of the total growth during last decades. About 84% of the world's production area and 83.4% of the world's overall production of cowpea is from Africa, with over 80% of African production in West Africa. It is estimated that 200 million children, women and men in West Africa consume the seeds daily (CGIAR, 2017). India is one of the largest pulse producers in the world accounting 23.02 million tons in 2019 including cowpea (Wikipedia, 2020).

Aside from being used as food, the plant parts have great demand in pharmaceutical industries. The secondary metabolite of cowpea helps in maintaining heart and cardiovascular system. The dietary fibers help in lowering cholesterol levels. The antioxidants act as an anti-inflammatory agent and build a health immune system. The lignin content of seeds keeps a number of fatal diseases like stoke and hypertension etc. The low fat content extremely beneficial for weight loss; high protein content accelerates the process of skin repair and help in keeping healthy and also useful in eliminating urination problems (D'Souza, 2019).

The literature survey reveals that stored seeds of *Vigna unguiculata* (L) Walp known to carry several fungal pathogens which invade cowpea grain during storage causing seed rotting, mycotoxin contamination and loss of seed viability, alter physio-chemical properties of the seeds, losses seed weight, germination potential, medicinal properties, and discolouration (Dawar, et al., 2015; Khare et al., 2016; Taylor and Ngaujah, 2016; Enyiukwu and Maranzula, 2017; Zanjare et al., 2020). Many workers have reported the association of species of *Fusarium, Aspergilli, Penicilli Rhizopus, Rhizotonia solani* with seeds of this crop (Mogle and Maske, 2012; Makun et al., 2012) and some of them are seed transmitted. The seeds infection can severely affect seed viability and seedling growth, and contribute to poor seedling vigour ultimately leading to poor crop performance and reduced productivity in terms of both quality and quantity (Enyiukwu and Maranzula, 2017).

It seems worthwhile considered that data on the diversity of fungal species of Nagpur would be a great importance for predicting the extent of Pre and Post infection and also might be of some use in future architecting bio-control to the seed deterioration and storage loss. It is very long that no investigations on bio-diversity of seed-borne fungal flora of cowpea during storage are carried out pertaining to the area of Nagpur. Keeping this in view, a survey on diversity of fungal flora associated with stored seeds of cowpea (*Vigna unguiculata* (L**.)** Walp) of Nagpur is undertaken.

MATERIAL AND METHODS

The stored seeds of *Vigna unguiculata* L.Walp has been selected for conducting the experiment as seeds of this crop are edible, providing high content of protein, minerals, fibers and low content of fats in addition to sufficient quantity of calcium, iron, vitamin B_1, vitamin B_2, vitamin C, phosphorus essential in daily human diet. In present investigation, after collection of 20 seed samples from cultivators and retailors of each

tehsil of Nagpur district were screened preliminary for apparent deformities employing dry examination technique (CMI, 2010). A randomly selected four hundred seeds from a composite of seed samples were screened for prevalence of seed-borne fungal pathogens employing standard blotter and agar plate technique as recommended by International Seed Testing Association (ISTA, 2020). Two hundred seeds without pretreatment were screened for detection of external seed borne while same count of seeds pretreated with aqueous solution of 0.1% mercuric chloride were placed to sterile petri plate containing semi-solid agar nutrient sterile medium composed of peeled potato (400gm-l), dextrose (20gm-l**)** and agar (20gm-l**)** in a liter of distilled water for isolation of internal seed borne fungal flora. After incubation for seven days in B.O.D incubator at 25±2°C under alternating cycles of 12 hours light and darkness, all untreated and pretreated seeds in petri plates were examined for fungal growth appeared on seeds surfaces. The seed borne mycoflora was identified with the help of colony colour and sporulation type. Fungal count and infestation level on untreated and pre-treated seeds have been recorded as a percentage of infested seeds in a sample following a technique reported earlier (CMI, 2010). The seed borne isolates were purified, sub-cultured and maintained on Czapek's Dox agar nutrient medium in sterile tube slants and species were identified on the basis of micro- & macro morphology; reverse and surface coloration of colonies grown in Czapek's medium (CMI, 2010) and finally authenticated by authority.

RESULTS AND DISCUSSION

Vigna unguiculata (L) Walp is one of the most ancient human food source, cultivated around the world for seeds from legume (Gitaitis and Nilakhe,1982). It is a store house of protein, carbohydrates and vitamins. This crop is attacked by diverse group of fungal pathogens causing several diseases. Majority of fungal pathogens are reported to grow on stored seeds as internal seed borne causing physiological damage to the seed. It causes loss of crop productivity that adversely affect economy of poor farmers (Srivastava et al.,2011). Literature review indicated that diversity of fungal flora adhering to seed surface of cowpea have not been from the Nagpur district of Maharashtra states hence an attempts have been made to study the same from Nagpur.

In the present investigation a count of 21 fungal pathogens of diverse group classified under 12 genera were confined on the seed surface indicating high level of fungal infestation of seed in storage condition (Table 1). Ascomycota dominated with 35.7% exhibiting highest count of fungal isolates followed by Deuteromycota contributing 35.5% isolates of the total population. Zygomycota contributed 21.4% isolates while Oomycota had least count. Spores of Basidiomycota did not appear on the seed surfaces of cowpea (Fig.1).

Oomycota had individual genera with single species. Zygomycota represented with 3 genera including *Cunninghamella elegans, Mucor pusillus* and *Rhizopus stolonifer* with single species.

Deuteromycota represented a population of 6 species belonging to 5 genera. Out of these, two species has been reported for *Curvularia*, while the others had single species.

Ascomycota had a fungal population of 11 species representing 3 genera. Out of this 11 isolates, a count of 9 species representing to the genus *Aspergillus* and other 2 genus *Penicillium* and *Phoma* is single species (Table 1).

Both blotter and agar plate technique are recommended for seed health testing and these two techniques are standardized from time to time by ISTA (2020) for accuracy. A fungal population of total 11 isolates belongs to 6 genera of diverse fungal group confined to cowpea seeds on blotter paper as well as agar plate as both external and internal seed borne pathogens. Altogether 5 species representing 3 genera restricted to blotter paper as external seed borne pathogen while 5 isolates confined only to agar plate as internal seed borne pathogens (Table 1).

Among the total population, 21 species belonging to 12 genera were associated with seed coat of cowpea. *Aspergillus* dominated with 9 species, exhibiting higher count of species against other genera encountered to seed surface. The genus *Curvularia* is dominated with 2 species while remaining had single genus with single species (Fig 2).

The seed borne fungi include a very large and heterogeneous group of organisms that occupy position of great economic importance in agriculture.

They exhibit an enormous diversity in life history. The seeds sample of cowpea is infected by fungal pathogens. Ascomycota dominated with nearly half of the total fungal incidences, represented by 46.1% followed by Deuteromycota contributing 27.9% incidences. Zygomycota had 21.2% while Oomycota 4.8% incidence (Fig 1).

Table 1: Percent incidence of fungal contaminants in storage on seeds of *Vigna unguiculata* (L)Walp

S. No	Name of fungal isolate	Frequency (%)of fungal incidence		Total Frequency	% over total incidence	
		Blotter	Agar		Species	Genus
A.	**Oomycota**	6 (3.6)	2(1.2)	8(4.8)	4.8	4.8
1	*Phytophthora infestans* de Bery	6	2	8	4.8	4.8
B.	**Zygomycota**	23(13.9)	12(7.3)	35(21.2)	21.2	21.2
2	*Mucor pusillus* Link	6	2	8	4.8	4.8
3	*Rhizopus stolonifer* (Eh.Ex.Rr.)Lind	15	10	25	15.2	15.2
4	*Cunninghamella elegans*	2	-	2	1.2	1.2
C.	**Ascomycota**	40(24.2)	36(21.8)	76(46.1)	46.1	46.1
5	*Aspergillus amstelodomi*(L.Mangin) Thom	2	-	2	1.2	41.3
6	*A. flavus*Link.	6	6	12	7.3	
7	*A. fumigates* Fres.	8	4	12	7.3	
8	*A. japonicas* Saito.	-	8	8	4.9	
9	*A. niger* Van Tieghen	8	8	16	9.7	
10	*A. oryzae* E.Cohn	6	4	10	6.1	
11	*A. phoenicis* (cord)Thom.	4	-	4	2.4	
12.	*A. sulphureus*(Fres.)T&C	2	-	2	1.2	
13	*A. terreus* Thom	2	-	2	1.2	
14	*Penicillium oxalicum* Thom.	-	6	6	3.6	3.6
15	*Phoma spp.*	2	-	2	1.2	1.2
D.	**Basidiomycota**	-	-	-	**-**	**-**
E.	**Deuteromycota**	24(14.54)	22(13.33)	46(27.87)	27.9	27.9
16	*Alternaria Cassiae*	-	6	6	3.6	3.6
17	*Cladosporium cladosporoides* (F) de Vries	-	4	4	2.4	2.4
18	*Curvularia lunata* (Wakker) Boedijn	10	4	14	8.5	14.6
19	*Curvularia ovoides* (H&W) Munt.	8	2	10	6.1	
20	*Fusarium solani* (Mert)APP.&Wollenw	-	4	4	2.4	2.4
21	*Helminthosporium tetramera* McKinney	6	2	8	4.9	4.9
	Total fungal incidence	93	72	165		99.9
	Per cent total incidence	56.4%	43.6%			

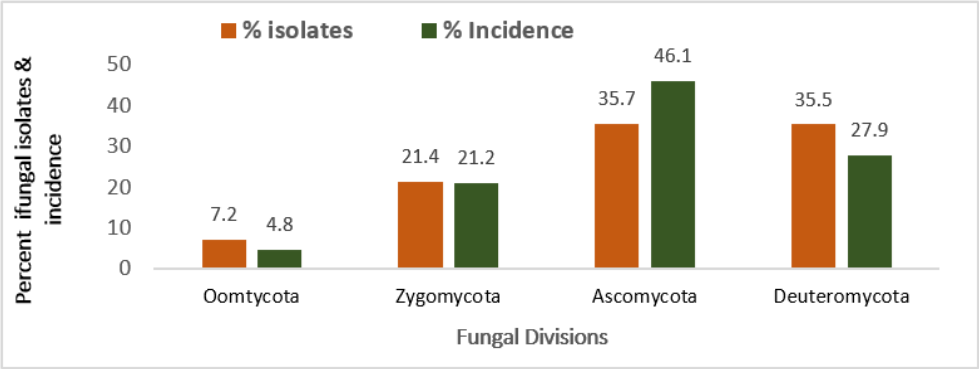

Fig. 1 : Percent division wise distributions of fungal isolates & incidence on seeds of *Vigna unguiculata* L

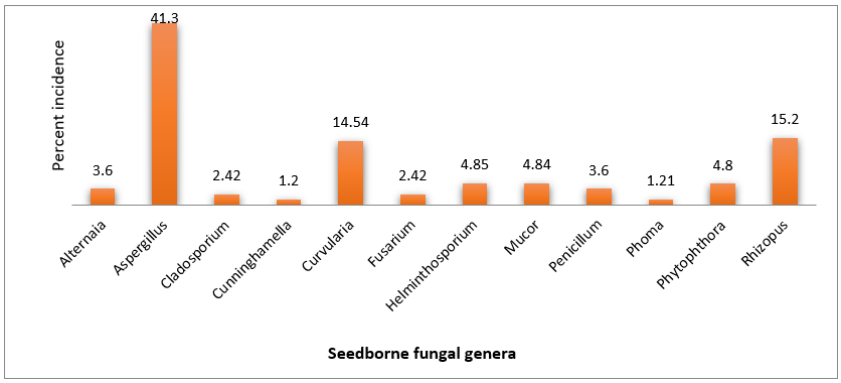

Fig.2: Genera wise %incidence of fungal flora on stored see

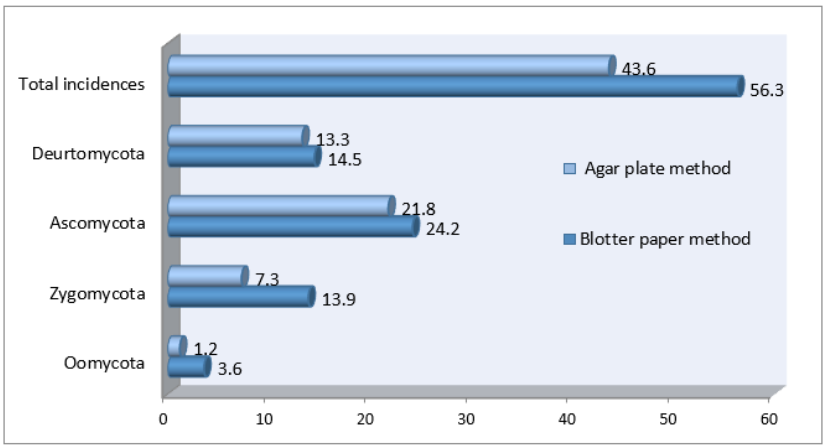

Fig. 3: Comparison between blotter paper and agar plate technique concern to % incidence of fungal pathogen on seed

Ascomycota exhibited higher level of infection on seed surface of cowpea. *A.niger* was dominated with 9.7% incidences of this fungal group. The isolates *A. flavus* and A. fumigates had moderate,7.3% incidence. The frequency of incidence ranged between 4.9 to 6.1% confined to *A.oryzae, A. japonicus* and *Penicillium oxalicum* (Table 1).

Deuteromycota had second higher level of infestation. *Curvularia lunata* was dominated with 8.5% incidence exhibiting highest fungal incidence compared to other isolates of this group. The infestation level varied between 3.6 to 6.1% for *Alternaria cassia, Curvularia ovoides* and *Helminthosporium tetramera.* The isolates *Fusarium solani* and *Cladosporium cladosporoides* had low level of incidences (Table 1).

Oomycota had only isolates *Phytophthora infestans* with 4.8% incidence while member of Zygomycota, *Rhizopus stolonifer* was dominated with 15.2% incidences exhibiting higher level of incidences (Table 1).

The seed coats of the cowpea seemed to be infested with variable fungal propagules. The incidences of all the fungal pathogens detected by standard blotter and agar plate technique were summarized to be 165 percent. Out of the total, 56.3% incidence was recorded on blotter paper while 43.6% incidences were observed on agar plate (Fig 3).

Ascomycota dominated with 24.2% and 21.8% followed by Deuteromycota with 14.5% and 13.3%; Zygomycota contributed 13.9% and 7.3% while 3.6% and 1.2% fungal incidences was recorded for Oomycota on blotter paper and agar plate respectively (Fig 3). However, higher incidences of fungal isolates were noticed on blotter paper from seed sample of cowpea over agar plate (Fig 5). These result coincide with the finding of earlier reports from other region of the country. Recently, Saskatchewan (2013) recorded higher frequency of fungal pathogen from seed of pulses on blotter paper over agar plate. Several other investigators reported similar finding by blotter test from infested stored seeds involving oil seeds (Anwar et al.,2013).

An individual genus of Ascomycota, *Aspergillus* contributed 41.3% incidence, which was more than one quarter of the total incidences followed by *Rhizopus*, contributing 15.2% incidence. *Curvularia, Helminthosporium* and *Mucor* exhibited significant level of infestation. The isolates of genera *Alternaria, Penicillium* and *Phytophthora* exhibited moderate infestation while others had little to mild infection (Table 1).

Ascomycota contributed 46.1% fungal incidences over total incidence followed by Deuteromycota 27.9% (Fig 4). The prevalence of maximum species confined to genus, *Aspergillus* contributing greatest percent incidences over total incidences. *Rhizopus stolonifer* is isolates on external as well as internal seed borne contamination (Fig 6). It is in agreement with Shripukar and Wahegaonkar (2012) who reported comparable higher count of species of *Aspergillus* such as *A. niger, A.terreus, A.fumigates A. flavus*from maize seeds. These results are in confirmation with earlier finding in *Solanum melongena* L (Bhajbhuje, 2013).

The dominant fungal genera in Deuteromycota are *Curvularia, Helminthosporium* and *Alternaria*. Bhajbhuje (2014) reported predominant of *Alternaria solani, Aspergillus flavus, Curvularia lunata* and *Helminthosporium tetramera* on maize seed.

The both standard blotter and agar plate test varied with nature of fungal flora. The member of Oomycota and Zygomycota develops more profusely on agar plate possibly because they requires softer medium rich in moisture for their establishment and growth. Among the seed health test techniques, standard blotter method was proved comparative superior over agar plate method to the fungal pathogen isolation. D'Souza, (2019) point out that in using the agar plate method, the quick growing saprophytes adhering to the outer seed coat may be troublesome to detect interval slow growing pathogen. These variations may possibly attributed to the prolonged incubation that might lead to the development of deep seated infection (Bhajbhuje, 2014; Zanjare et al., 2020). The mycological analysis of the infected and disinfected seeds gave only general information of the inner seed infection, the fungal propagules present on the surface of seed do not penetrated the inner tissue, this information is not precise, that the starting point to determine proper strategies of seed treatment.

The fungal isolates belong to genera *Aspergillus* of Ascomycota as well as *Curvularia, Helminthosporium and Alternaria* of Deuteromycota contributed as major component on cowpea seed (Table 1); Deuteromycota had comparatively higher count of fungal isolates associated with stored seed but Ascomycota has greatest frequency of fungal incidences on cowpea seed followed by Deuteromycota (Fig. 1). It may possible that prevalence of greatest count of fungal propagules associated with seed coat with their higher incidences. Member of this group is known to facultative parasite on crop plant as well as involved as saprophyte in biodegradation of seeds and debris of plants and animals origin (Jyoti and Malik, 2013). It was interesting of Basidiomycota did not appear on cowpea seeds as fungal pathogens of this group are mainly obligate parasites of other crop plants.

The report of the study shows that Ascomycota genera, *Aspergillus* were the highly dominated on cowpea seeds are among the most abundant and widely distributed organisms on the globe (Bhajbhuje, 2013; Khare et al., 2016). The genus *Aspergillus* are obligates saprophyte and survive in the environment without causing diseases, *Aspergillus flavus, A.fumigatus, A. niger, A.oryzae* had the highest count of occurrences. These species are commonly isolates from seeds and other substrates (D'Souza, 2019) and brinjal seeds (Bhajbhuje, 2014).

Aspergillus niger has produce ochratoxin-A. *Aspergillus flavus* secretes aflatoxin B_1, B_2,$G_1$and $G_2$and other toxic compound like cyclopiazonic acid, kojic acid, asertoxin, aflatrem and Aspergillus acid. Helminthosporium produced Helminthisporin, four different HC toxin; *Curvularia lunta* produces -2 methyl-(5- hydroxyl methyl) Furan-2 carboxylate. Fusarium solani were repoted to cause Keratitis and also associated with wound and infections of the eyes and fingernail (EFSA,2011). The other fungal pathogen viz., *Mucor pusillus*secrets citrinin and penetrem-A; All these toxins are Known to create physiological disorder to consumes (EFSA,2011). The cowpea are affected with several fungal diseases including root rot, damping off, *Pythium*, *Rhizoctonia*, *Fusarium wilt* and *Fusarium oxysporum*. During seedling emergence, the pathogen transmit from seed to seedling causing premature defoliation, multifold loss to both pre and post harvest crop that adversely affected economic of poor farmers (Srivastava et al., 2011).

The result of prevent study indicated that cowpea seeds are attack by various fungal contamination. Some of these fungi had been reported in various stored seeds. The quality of seed at the time of storage, environmental factors, moisture content, relative humidity, temperature of storage, duration of storage and biotic agents are responsible for its contamination. The growth of isolates fungi results in change the cellular contain, metabolic and chemical alternation, chromosome aberration and DNA damage ,enzyme degradation, loss of membrane, lowering of ATP, loss of the protein and sugar contain, change in nutritive quality and accumulation of toxic substance which lead to spoilage of seeds (Jyoti and Malik, 2013).

On the other hand, the fungal spore in seeds are also danger to the public health since their metabolites (mycotoxins) produced in seed may lead to serious clinical condition in the consumer (EFSA. 2011). More than 300 fungal metabolite are reported to be toxic to man, animals and post serious health hazardz (Lew-Smith, 2013; EFSA, 2011)

The fungal isolates in cowpea are xerophilic moulds such as *Aspergillus* and *Penicillin* of Ascomycota as well as *Alternaria*, *Curvularia*, *Fusarium*, *Helminthosporium* of Deuteromycota(Bhajbhuje , 2014) Infected seeds with pathogen often fail to germinate , transmit diseases from seed to seedling and from seedling to growing plants , thus seeds born diseases cause enormous loss to the crop and also the viability period (Lew-Smith, 2013; Khare et al., 2016).

CONCLUSION

The seeds of *Vigna unguiculata* L. is store house of good quantity of protein, carbohydrates and vitamins and a low fat. They are prone to attack by various seed borne fungal pathogens that transmit from seed to seedling and spread diseases as well as deteriorate seeds. The results of present study indicated that cowpea seed are affected by various fungal contaminations as a result of improper storage environment. The practices associated with the quality of seeds at the time of storage, environmental factor, biotic agent are responsible for contamination which leads to spoilage of seed. Many fungal isolates involved in seed deterioration of cowpea are *Aspergillus* and *Penicillum* of Ascomycota as well as *Alternaria, Curvularia, Helminthosporium* of deuteromycota sowing of deteriorated seeds, increase chance of pathogen transmission to a new crop. The toxic metabolites secreation on the seed is also responsible for spoilage of seed. The mycotoxin induces micro mutation which causes carcinogenic disorder. The metabolites are toxic to animals and plants, hence the fungal spores on seeds are suggested to dangerous for public health.

Seeds are important input of crop production so that fungal free healthy seed are considered as a vital factor for desired plant and good economic harvest. It was concluded that the seeds of cowpea are attack by several fungal pathogen on seed surface, the fungal pathogen damage the seeds and also the nutritional components of the seeds. The internal seed borne pathogen involve in cowpea diseases. So it was important to develop the strategy against the seed borne diseases. Only high quality seed respond better to all inputs. Thus seeds can be stored under temperature and relative humidity at very low cost, without deterioration in quality for a period of one or more season is of immense importance for farmer. The farmer is advised to use scientific method for seed storage.

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Conflicts of Interest: The author declares no conflict of interest

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