GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY
© 2004 - 2018 Society For Science and Nature (SFSN). All rights reserved
www.scienceandnature.org

ANALYSIS OF FOOD GRAIN SPOILAGE FUNGI AND AFLATOXINS IN RAW AND PARBOILED RICE SAMPLES COLLECTED FROM CHENNAI, TAMIL NADU

K.Vadamalai Krishnan, K.Sureshkumar and P. Jayaraman*
PG and Research Department of Botany, Government Arts College for Men (Autonomus)
Nandanam, Chennai 600035, Tamil Nadu, India
*Corresponding author e-mail: jayaramannp@gmail.com

ABSTRACT
Since rice is the staple crop of India, the present study is focused on the milled rice such as raw rice and parboiled rice to study its contamination with storage fungi and aflatoxins B_1 collected from Public Distrinution System (PS) shops of Chennai, Tamil Nadu. Twenty five rice samples (10 raw rice and 15 parboiled rice) were collected for the analysis of moisture content, food grain spoilage fungi and the presence of aflatoxins. The moisture content of the rice samples were determined by hot-air oven drying method and found in the range of 5.0% to 10.4% with the average of 10.09 % for both raw and parboiled rice samples. For the analysis of food grain spoilage fungi through direct plating of grains on Czapak's Dox Agar (CDA) plate, it was found that the fungi mainly comprising different species of *Aspergillus and Pencillium*. Few other fungi like *Mucor, Rhizophus, Pyricularia, Helminthosporium and cladosporium* also were observed in very less numbers in one or other rice samples. The individual species of fungi such as *Aspergillus niger, A. glaucus, A. flavus, A. terreus, A. nidulans, A. fumigatus, A. candidus, Penicillium citrinum, P. Funiculosum, P.Chrysogenum* and *P. tardum* were encountered. The quantitative pattern of fungi showed that the raw rice samples showed higher number colonies of individual fungi when compared with parboiled rice samples which had in lower numbers. Among 25 samples of parboiled and raw rice samples analysed for the presence of aflatoxins, 2 each from raw and parboiled rice samples were found positive for aflatoxin B_1 with the range of 2 ppm to 5 ppm as very low level of contamination. For the screening of aflatoxigenic *A. flavus*, 8 strains were found toxigenic among 12 strains of *A. flavus* isolated from rice samples.

KEYWORDS: Parboiled rice, Raw rice, Aflatoxin B_1, storage fungi, Moisture content, *Aspergillus, Penicillium.*

INTRODUCTION

Post-harvest losses during handling and storage in agricultural commodities like cereals, pulses, millets and oilseeds having been a major problem in tropical countries like India and the losses were estimated into several million rupees. The storage fungi and mycotoxins are the major causes of loses in agricultural commodities and it products from harvesting to storage. These are not only affect the nutrient quality and also poisonous due to secretion of toxic substances to human and veterinary. Among the food grains, the rice is cultivated as a major food crop in Tamil Nadu and it is used for many food preparations in our daily life. The contamination and spoilage of food grains especially in rice has reported by various scientists from the field during maturation, harvest, handling operation, milling process and storage in godowns to distribution in various consumers. Among these, the storage of grains is an important phenomenon for growth and development of microorganisms due to favorable environmental condition. The reason for such storage losses in rice are temperature, moisture, damages of grains during harvesting, mites, weevils, pests, microorganisms like fungi and bacteria. Among these factors, microorganisms are the chief causative agents which comprised of several species of *Aspergillus*, few species of *Penicillium, Mucor and Rhizophus* which are commonly referred as Storage Fungi (Christensen and Kaufmann, 1969). The spoilage of rice including nutritive losses and contamination by microorganisms like fungi is occurred due to various environmental and other factors in different forms of rice as raw rice and parboiled rice from warehouses and various storage places before being distributed to consumers are known. The main effects of these food grain spoiling fungi are (i) Quantity losses like damage and inferior physical appearance of grains (ii) Qualitative Biochemical changes including nutrient losses like protein, carbohydrate, lipids, vitamins and minerals; reduction of germinability of grains and (iv) secretion of secondary metabolites called as mycotoxins including aflatoxins which are highly toxic to human and animals especially cattle. The production of mycotoxins including aflatoxins are occurring due to higher growth of storage fungi comprised of different species of Aspergillus especially *Aspergillus flavus* which is a poisonous substances to produce liver cancer or hepatocarcinoma.

Mycotoxin contamination in certain agricultural commodities has been a serious concern for human and animal health. Mycotoxins are substances produced mostly as secondary metabolites by filamentous fungi that grow on seeds, grains, and feed in the field, or in storage. The major mycotoxin-producing fungi are species of Aspergillus, Fusarium, and Penicillium. Aflatoxins, fumonisins, trichothecenes, ochratoxins, cyclopiazonic acid, patulin, deoxynivalenol, zearalenone, citrinin,

gliotoxin, and sterigmatocystin are some of the important mycotoxins. This paper reviews the mycotoxigenic fungi, their levels of mycotoxins, and their management by using botanicals, microbiologicals, and cooking methods in rice. The data from detailed investigations on rice seeds and grains help to provide safe grains for consumption and export, and prioritize future research programs (Reddy *et. al*., 2008).

An investigation carried out in German supermarkets led to the isolation of 191 different species of fungi from 185 separate samples; these consisted of 26 aspergilli, 118 penicillia, 19 members of mucorales and 28 other species (Boesenberg and Eberhardt, 1969). The food grains widely investigated for storage fungi and mycotoxins include wheat, maize, sorghum, rice, barley, oats and millets and various oilseads. Fungi occurring in rice grains include different species of *Aspergillus* and *Penicillium* and to some extent field of fungi. As early as 1952, Del Prado and Christensen reported the occurrence of Aspergilli and Penicillia in stored rice samples from Llouisiana and Surinam. Various scientific reports from show on the contamination of both Aspergillus and Penicillium in different rice samples were equally dominant (Naito, 1984; Udagawa, 1959; Kurata *et al*., 1968; Miyaki et *al*.,1969; Tsuruta, 1973; Tsuruta and Saito, 1980; Vasanthi Siruguri, 2012). Alkenz *et al.*,2015 reported in 24 samples of couscous, macaroni, wheat flour and rice from Libya and isolated 113 isolates of fungi belonging to nine genera such as *Penicillium*, *Aspergillus*, *Fusarium*, *Paecilomyces*, *Alternaria, Rhizopus*, *Mucor, Scopulariopsis* and *Cladosporum* which are known as main producer of mycotoxins especially *A. flavus* known to produce aflatoxins, *Aspergillus niger, Aspergillus carbonarious*, *Penicillium chrysogenum* and *Penicillium verrucosum* known to produce ochratoxin and *Fusarium oxysporum* and *Fusarium chlamydosporum* known to produce fumonisins and trichothecenes. Onyeze Rosemary *et al.*, 2013, collected two type of samples of spoilt corn, red (treated) and white were taken from the store and field respectively for investigation in order to ascertain the fungi and found with 5 genera such as Mucor spp 6%, Aspergillus spp. 9%, Rhizopus spp. 15%, Penicillium Spp. 33% and *Fusarium* Spp. 36% which were the microorganism spoilt the corn very extensively. Wajiha Iram *et al.*, 2018 screened aflatoxigenic fungi from stored maize samples and their growth inhibition by using herbal treatment. Among 6 species of genera Aspergillus, they isolated 45 stains of *A. flavus* from different samples and found all of them were able to prduce aflatoxin B_1 invitro at varying level of concentration. Further they evaluated the aqueous extracts from ten medicinal plants for their potential to restrict the mycelial growth of aflatoxigenic *A. flavus* isolates and found Leaf extract of Eucalyptus citriodora showed highest growth inhibition (100 – 98%) which was followed by *Trachyspermum ammi* seeds and leaves extract of *Ocimum basilicum i.e*., 90.8–91.8% and 82.8– 87.7%, respectively.

Fungi associated with seeds of five cultivars of rice were isolated by Uma and Wesely, 2013 and isolated the fungi namely *Aspergillus flavus, A. niger, Penicillium citrinum, Alternaria padwickii* and *Rhizopus oryzae* from the contaminated surface of un-milled rice grain. Chowdhury *et al.,* 2014 analysed the Rice stored in indigenous storage containers viz., i) earthen jar (EJ), ii) coaltar coated EJ, iii) seed mixed with *neem* leaves (*Azedirecta indica*) in EJ, iv) seed mixed with *Biskatali* leaves (*Polygonum hydropiper*) in EJ, v) biscuit tin, vi) drum, vii) hessian bag (HB), viii) hessian bag with polythene cover, ix) *dole* and x) cowdung coated *dole* for four months in the laboratory and observed the changes of seed such as moisture content, germination, insect and pathogen attack, protein and starch contents over 120 days of storage. In case of almost impermeable containers like biscuit tin, drum and hessian bag with polythene cover, seed moisture increased significantly. Moisture percentage of the seeds of biscuit tin, drum and hessian bag with polythene cover was 12.78, 12.95 and 12.88 %, respectively. Germination percentage of seeds in all impermeable containers was above 80 %. Field fungi like *Alternaria, Curvularia, Cladosporium* though observed initially, but in the last pathological test *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. were found predominantly. Insect attack was severe in easily permeable containers. Permeable containers containing the leaves of *neem* and *biskatali* restricted insect and fungal attack. Starch content of seed was 77% at 60 days of storage and decreased to 75 % at 120 days of storage. The overall observation suggests that, biscuit tin and drum could be used for small scale storage and hessian bag with polythene cover for bulk storage of rice seed for a certain period.

The occurrence of storage fungi in rice is extensively studied in University Botany Laboratory, University of Madras by Indira Kalyanasundaram and her Co-workers since 1975 in various ways and they arrived many conclusions. The significant findings were the occurrence of storage fungi such as different species of *Aspergillus* and *Penicillium* wre frequently found in the stored rice from various godowns and distribution systems like various shops as well as in standing rice crops. They found that the group of species of *Aspergillus* and *Penicillium* predominantly *Aspergillus glaucus, A. flavus, A. candidus, A. niger, A. fumigatus, A. nidulans, A.versicolor, A.ochraceus, Penicillium citrinum, P. funiculosum and Mucor and Rhizopus* are occurring in stored as well as standing rice grains. Among the species of storage fungi, *Aspergillsu flavus* is the potent mycotoxins producing fungi growing in grains at higher moisture level and produce aflatoxins significantly. The mycoto xins especially Aflatoxins are hazardous and create health problems (carcinogenic) in human as well as animals. The major mycotoxin-producing fungi are species of *Aspergillus*, *Fusarium*, and *Penicillium*. Among these, *Aspergillus flavus* sub sp. *parasiticus* is a potent aflatoxin producing fungi occurred frequently in stored food grains as well as in the freshly harvested grains before storage. The natural occurrence of aflatoxins in rice bran and its products were confirmed by Jayaraman and Kalylanasundaram, 1990; Jayaraman and Kalyana sundaram, 2009), Wajiha Iram *et al*., 2018. The contamination of aflatoxin B_1 in rice bran, de-oiled bran and cattle-feed and M_1 in milk were studied and found low to moderate level of aflatoxin contamination (Jayaraman, 1991; Jayaraman and Kalyanasundaram 1994; Jayaraman and Kalyanasundaram 2009). This indicates it posses

health hazardous to man and veterinary animals if the food and feed is consumed continuously with high level of contamination with aflatoxins. Survey of contamination of storage fungi and aflatoxin B_1 in cattle feed was made by Jayaraman *et al*., (2017) and found 60% of the samples contaminated with aflatoxin B_1. The main disease cause in human and animals with aflatoxin contaminated food and feed is hepatic carcinoma or liver cancer. Hence, it is important to study the contamination of various and food and feed prepared with agricultural commodities including cereals, pulses and oilseeds as a raw material.

Therefore, the present study is proposed to find out the contamination by different species of storage fungi in rice and occurrence of aflatoxins due to the production by *Aspergillus flavus* in contaminated rice samples. The rice samples collected in the present study is from godowns, open markets and ration shops of Chennai city, Tamil Nadu. The methodology involved is simple microbiological technique in which the rice will be placed on agar media contain nutrients (Czapek's Dox Agar (CDA) contain high sucrose content) and the qualitative and quantitative pattern of storage fungi and their diversity is to be studied. All the samples of rice from both parboiled and raw rice will be extracted for aflatoxins and anlaysed by Thin Layer Chromatograph (TLC) method. The results of the present study will be interpreted and possibility of control methods through various techniques can be evolved.

MATERIALS AND METHODS

Collection of rice samples

Rice samples such as raw rice and parboiled rice from various sources like rice shops, PDS (Public distribution system) shops and open markets were collected in the present study from Chennai, Tamil Nadu. The rice samples about 200g in each lot were collected in pre-cleaned polythene bags (LDPE bags) and tied tightly with rubber bands.Different rice samples collected in polythene bags were labeled properly for date and source of sample which are kept in the laboratory for further analysis.

Determination of Moisture content

The moisture content of the individual rice samples were analysed by using hot-air oven drying method. This method involve in drying of rice samples in known weight of crucibles at 100 ^{o}C for 1hour period. The loss on drying of pre-weighed quantity of rice is calculated during drying in oven and the calculation of moisture present in the samples is done. Then the percentage (%) of moisture in rice is calculated. The procedure is repeated until constant weight is obtained after drying in hot-air oven.

Analysis of food grain spoilage fungi

Qualitative pattern

The qualitative pattern of food grain spoilage fungi otherwise called as storage fungi is analysed by agar plating method. The nutrient culture media for culturing of fungi from rice grains were Potato dextrose agar (PDA) and 50% sucrose containing Czapak Dox agar (CDA). The high sugar content used in the culture media of CDA is for harbouring storage fungi which is highly osmophilic from the nutrient environment (Rao and Kalyanasudaram 1983). The growth of individual storage fungal species were observed and identified to know the qualitative pattern of the fungi present in the grains. The different species of Aspergillus and Penicillium were identified according to standard methods and The manual of Aspergillus, The manual of Penicllia (Raper and Thom, 1965; Raper *et al*., 1949; Jayaraman and Kalyana sundaram, 1994) used in the present study.

Quantitative pattern

The quantitative pattern of individual species of food grain spoilage fungi from the rice grains analysed in the present study is observed as total number of individual fungal colonies grown in the agar plates plated with rice grain samples. The number of fungal species present in the grains also were counted as number of individual colonies of fungi and recorded as quantitative pattern. The percentage occurrence was calculated and expressed as percentage of fungal colonies.

Culture Media Preparation

For enumeration of storage fungi *i.e*., mainly the species of *Aspergillus* and *penicillium* which are osmophilic fungi, the standard procedure was used (Sheila *et al*., 1978) throughout the study. The high osmotic medium used was Czapek-Dox Agar containing 50% w/v sucrose *i.e.* 50% CDA (Rao and kalyanasundaram, 1983).

For the maintenance of cultures, test tube slants of normal Czapak-Dox agar with 3% sucrose (3% CDA) were used to isolate the individual species of fungi. These cultures were stored in the refrigerator after 7 day's growth and sub-cultured as and when required.

The composition of the medium was as follows:

Sucrose	500 g for (50%) or 30g for (3%)
Sodium nitrate (Na No_3)	3g
Dipotassium hydrogen phosphate (K_2HPO4)	1g
Magnesium Sulphate ($Mgso_4.7H_2O$)	0.5g
Potassium chloride ($Feso_4$.$7H_2O$)	0.5g
Ferrous Sulphate ($Feso_4.7H_2O$)	0.01g
Agar	20g
Distilled Water	1000 ml
pH adjusted to	6.5.

Stock solution (500 ml) of forty – fold strength of each of the salts namely dipottassium phosphate (20g), Magnesium sulphate (10g), potassium chloride (10g) and ferrous sulphate (200mg) were prepared separately in distilled water and stored. When required, 25 ml aliquots from each of the above solution of salts were made updo 400ml with distilled water, with the sodium nitrate and to the solution and mixed thoroughly. The molten medium was distributed in 500 ml conical flasks. After sterilization and before pouring plates, 25 units of strepto penicillin per ml of medium were added from a stock solution in order to suppress the bacterial growth.

To observe the specific characters of storage fungi for identification, the following media were used (Raper and Fennell, 1965).

3% CDA (Composition given as before)

Malt agar

Malt extract - 20g
Sucrose - 30g
Agar - 20g
Distilled Water - 1000 ml
pH adjusted to 6.5.

Inoculation and Incubation

The sterilized agar medium poured on petri-plate in the inoculation chamber and allowed to set the molten agar. Then the individual rice grains from each sample were plated (inoculated) on the agar medium after solidification of agar in the glass petri plates. The inoculated plates were labeled properly with date and sample details.

Then the rice grains inoculated agar plates were incubated at 30+/- 1^{o}C for maximum of one week period in the incubator. The incubation was continued until the growth of fungal colonies was completed.

Observation and identification

During incubation period, the agar plates grown with fungi were observed for its qualitative pattern i.e. identifying individual fungal species. The number of colonies formed on the rice grains inoculated was observed and identified by using standard methods after Raper and Fennell, 1965; Raper *et al*., 1949; Ellis, 1971). The quantitative pattern is calculated for the percentage infestation in rice grains based on the individual species of fungi colonized in individual grains.

Analysis of Aflatoxins

The following simple screening method for analysis of aflatoxins in rice is used for the present study. This includes the extraction of powdered rice sample for aflatoxins with suitable solvent as methanol and separation and identification by using Thin Layer Chromatography (TLC). The simple screening method by Seitz and Mohr (1972) using methanol as a solvent and separation by Thin Layer Chromatography (TLC). The process steps are outlined in the following text. First the Pre-weighed quantity (20g) of rice samples was powdered by using laboratory mixer and the fine powder taken for extraction. The powdered rice samples are extracted with 100 ml of methanol solvent for 30 sec. The extracted rice sample was separated by using hexane, acetonitrile and finally with methylene chloride by using a 250 ml separatary funnel.The extracted sample condensed in to smaller quantity and spotted on TLC plates. Then the spotted TLC plates with sample extract were developed with chromatogram solvent system as Benzene and chloroform (88:12). Then the developed TLC plates are air dried and observed under UV lamp at 365 nm for the flouresence of spots in blue colour which is identified as aflatoxins B_1 and B_2. The fluorescent spots in other colours like green indicates aflatoxin G_1 and G_2.The sample extract was chromatographed comparatively with standard aflatoxin B_1. The amount of aflatoxin B_1 is expressed as ppm level. The quantitative estimation of the aflatoxin from samples was made by the spectrophotometric method described by Nabney & Nesbitt (1965). Aflatoxin recovered in cold methanol from silica gel plate was read spectrophoto meterically at 363 nm and 420 nm and the OD values were taken for calculation.

Screening of toxigenic *Aspergillus flavus*

To test the fungal isolates for production of aflatoxin in vitro, the strains of *Aspergillus favus* were cultured in slants of an agar medium containing 2% yeast extract and 15% sucrose (YES). Toxins were extracted from the molten agar with chloroform and assayed by TLC using toluene: ethyl acetate: 90% formic acid (6:3:1) solvent system (Bullerman, 1974). The fluorescent spot in blue and green TLC under short wavelength indicates the presence or absence of aflatoxin and the toxigenic property of the *Aspergillus flavus* strains.

Statistical analysis

Analysis of rice samples for the above parameters was made in triplicate to avoid sampling and experimental errors. The values obtained for triplicate of experiments were statistically analysed for calculating mean value and standard deviation (SD) to expressed as mean (n=3) +/- Standard Deviation (SD).

RESULTS

Sample details

Totally 25 rice samples from both parboiled and raw rice were collected from various sources as local markets and public distribution system (PDS) of Chennai city for the present study. Among 25 rice samples, 15 were from parboiled rice and 10 from raw rice samples. The collection of sample was done in two slots and mentions as slot I and slot II. The details of samples collected from different sources in the present study are indicated in the Table 1 and Table 2.

TABLE 1. Details on collection of Raw and Parboiled rice samples (Slot I)

S.No	Samples details	Place
1	Raw rice	Royapuram
2	Parboiled rice	Washermenpet
3	Parboiled rice	Kasimedu
4	Parboiled rice	kodambakkam
5	Parboiled rice	T.nagar
6	Parboiled rice	Nandanam
7	Raw rice	Royapuram
8	Parboiled rice	Broadway
9	Parboiled rice	Tondiarpet
10	Parboiled rice	Besant nagar
11	Raw rice	Nandanam
12	Parboiled rice	Royapuram

13	Parboiled rice	Saidapet
14	Raw rice	Adyar
15	Raw rice	Vadapalani

TABLE 2. Details on collection of Raw and Parboiled rice samples (Slot II)

SI.No	Samples Details	Place of collection
1.	Raw rice	Kasimedu
2.	Raw rice	kodambakkam
3.	Raw rice	T.nagar
4.	Raw rice	Nandanam
5.	Raw rice	Royapuram
6.	Parboiled rice	Broadway
7.	Parboiled rice	Tondiarpet
8.	Parboiled rice	Besant nagar
9.	Parboiled rice	Nandanam
10.	Parboiled rice	Royapuram

Moisture content of rice samples

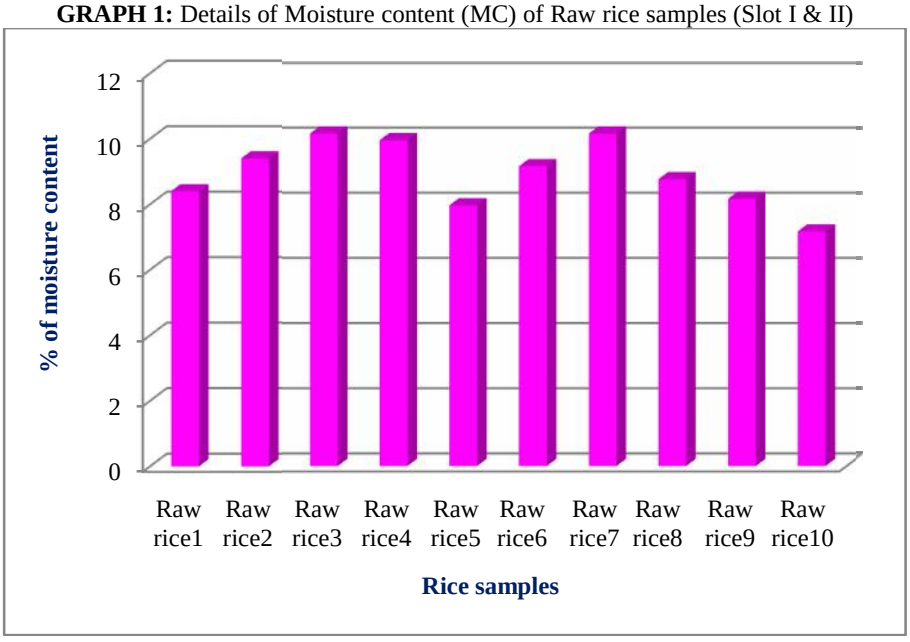
For the sake of convenience of comparing the results of moisture content, mycoflora and aflatoxin B1 content of samples from the slot I and slot II analysed in the present study, the raw rice and parboiled rice samples were categorised in a separate table and discussed in the following text.

For the analysis of moisture content by oven – drying method, the range of moisture content observed from 7.2% to 10.2% with an average of 8.96 % for raw rice samples. The parboiled rice samples showed from 5.0% to 10.4% with an average of 7.04% When comparing the parboiled rice samples with raw rice samples, the moisture percentage is lower in most of the parboiled rice (average of 7.04%) and higher in raw rice samples (average of 8.96%). The details of moisture content of different raw rice samples collected were given in Table 3 and Graph 1 and parboled rice samples in Table 4 and Graph 2.

TABLE 3: Details of moisture contents of rice samples (Slot I)

Sl. No	Samples detailes	Moisture content (%)
1.	Raw rice	8.40
2.	Raw rice	9.40
3.	Raw rice	10.20
4.	Raw rice	10.00
5.	Raw rice	8.00
6.	Raw rice	9.20
7.	Raw rice	10.20
8.	Raw rice	8.80
9.	Raw rice	8.20
10.	Raw rice	7.20

GRAPH 1: Details of Moisture content (MC) of Raw rice samples (Slot I & II)



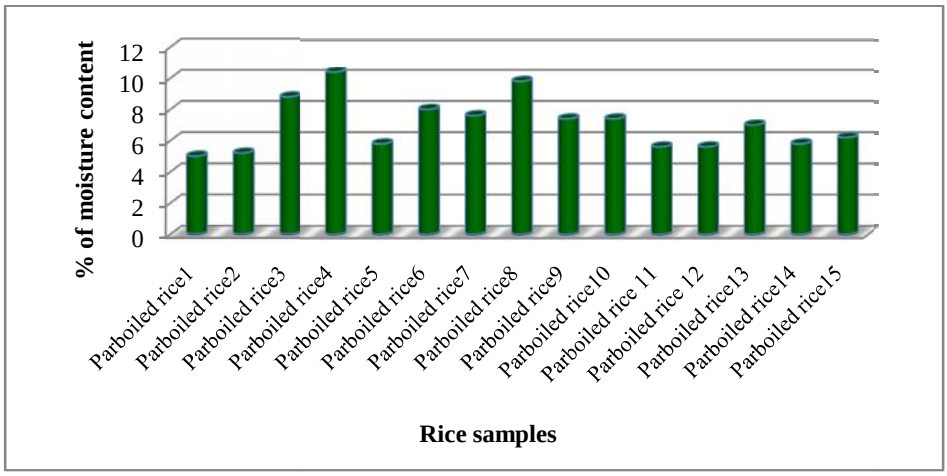
Analysis of fungi

Qualitative pattern

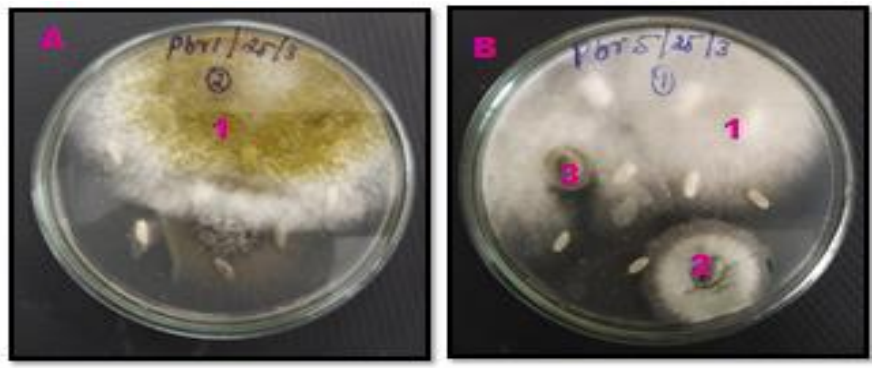
The storage fungi mainly comprising different species of *Aspergillus and Pencillium* were encountered in parboiled ad raw rice samples in varying numbers. Few other fungi like *Mucor, Rhizophus, Pyricularia, Helminthosporium and cladosporium* also were observed in very less numbers in one or other rice samples which were not considered significantly as food grain spoiling fungi. The individual species of *Aspergillus and Penicillium* such as *Aspergillus niger, A. glaucus, A. flavus, A. terreus, A. nidulans, A. fumigatus, A. candidus, Penicillium citrinum, P. funiculosum, P.chrysogenum, P. tardum, Mucor mucedo, Rhizophus stolonifer* and *Pyricularia oryzae* were encountered in the present study. The growth and morphological appearance of individual species of fungi are shown in Fig. 1 and Fig. 2.

TABLE 4. Moisture content (MC) of Parboiled rice samples (Slot I & II)

Sl. No	Samples detailes	Moisture content (%)
1.	Parboiled rice	5.00
2.	Parboiled rice	5.20
3.	Parboiled rice	8.80
4.	Parboiled rice	10.40
5.	Parboiled rice	5.80
6.	Parboiled rice	8.00
7.	Parboiled rice	7.60
8.	Parboiled rice	9.80
9.	Parboiled rice	7.40
10.	Parboiled rice	7.40
11.	Parboiled rice	5.60
12.	Parboiled rice	5.60
13.	Parboiled rice	7.00
14.	Parboiled rice	5.80
15.	Parboiled rice	6.20



GRAPH 2: Details of Moisture content (MC) of Parboiled rice samples (Slot I & II)



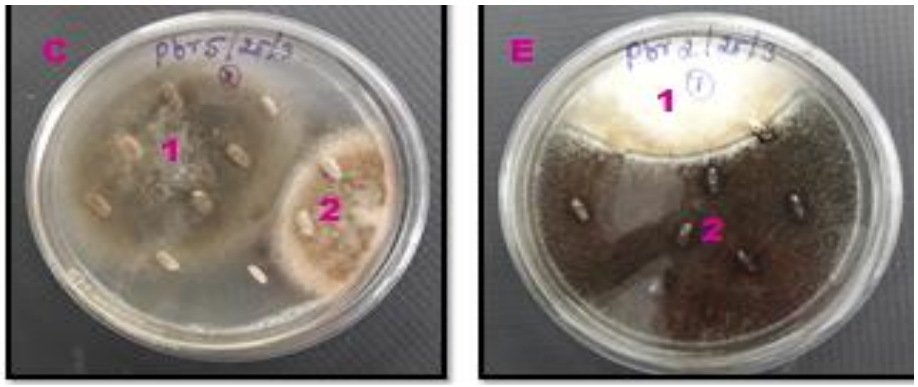


FIGURE 1. The growth and appearance of Fungi in Parboiled rice samples

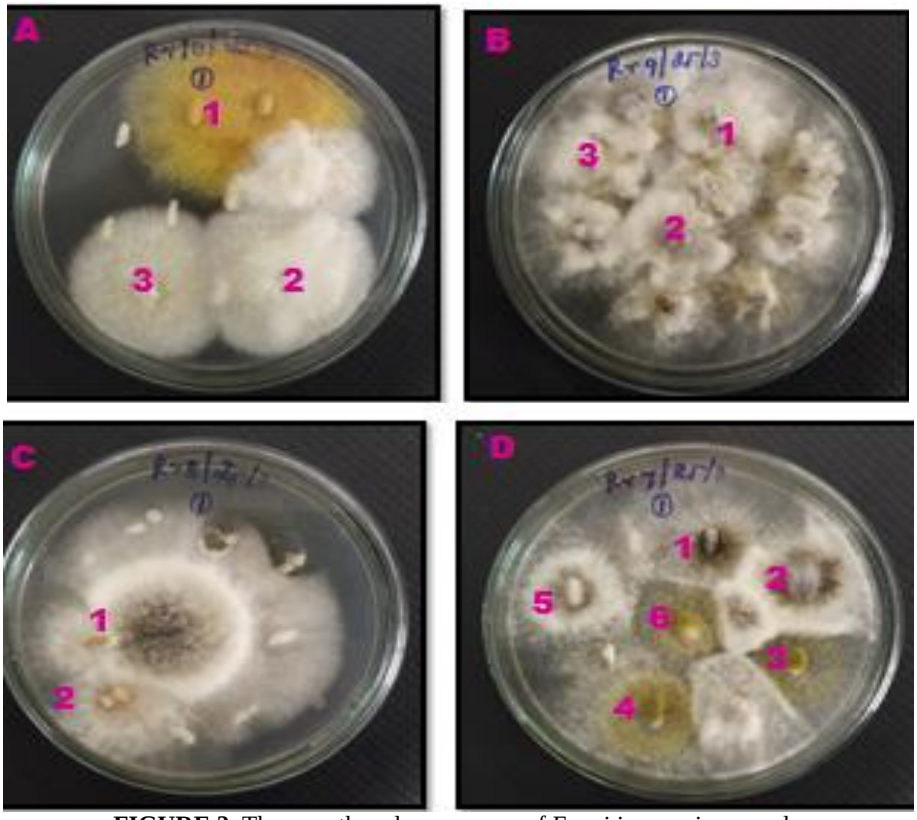


FIGURE 2. The growth and appearance of Fungi in raw rice samples

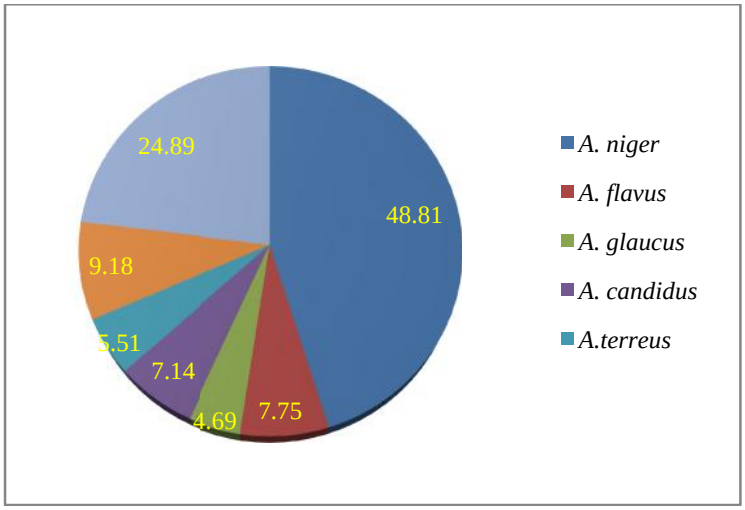
Quantitative pattern of fungi

The quantitative picture of individual species of fungi grown in individual grains of raw rice and paroiled rice were counted and total number of fungi was calculated. Then the percentage of occurrence of individual fungi was calculated and interpreted throughout study. Among 25 samples of raw rice (10) and parboiled rice (15) samples analysed for the presence of storage fungi, raw rice samples found with more number of fungal contamination than parboiled rice samples. Among the individual species of fungi, *A. niger, A. flavus, A. glaucus and A. terreus* were frequently occurred in both raw rice and parboiled rice samples. *Aspergillus candidus* found very rarely in rice samples. However, the *Penicillium* species (*P.citrinum* and other species) occurred constantly equal to the occurrence of *Aspergillus* species.

Among the individual species of fungi occurred in raw rice, *A. niger* showed higher population followed by *Penicillium spp., A.flavus, A.candidus* and *A.terreus* respectively. Though Mucor sp. occurred in almost all the grains, its contamination is very rapid in agar plate. The details of occurrence of individual species of fungi in the raw rice samples are shown in Table 5 and the overall population of fungi is depicted in Graph 3.

TABLE 5. Percentage occurrence of individual species of fungi in Raw rice samples

SI.No	Sample details	A.n	A.f	A.g	A.c	A.t	P. sp.	Mucar sp.	Total
1	Rr 1	58	8	8	13	0	13	0	100
2	Rr 2	5	0	0	2	1	2	0	10
3	Rr 3	0	1	1	0	0	1	0	3
4	Rr 4	1	0	0	1	9	0	0	11
5	Rr 5	10	0	0	0	0	0	0	10
6	Rr 6	3	2	2	4	2	1	0	14
7	Rr 7	31	15	12	15	15	6	0	94
8	Rr 8	55	0	0	0	0	22	22	99
9	Rr 9	37	12	0	0	0	0	50	99
10	Rr 10	0	0	0	0	0	0	50	50

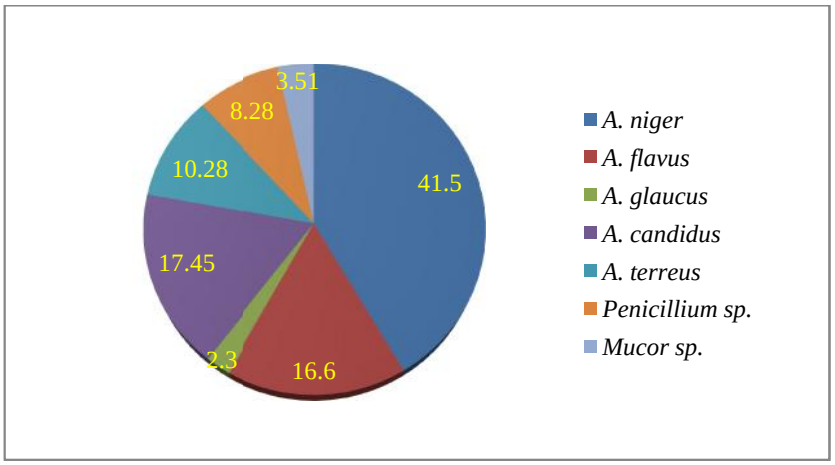


GRAPH 3. Percentage ooccurrence of individual species of fungi in raw rice samples

The overall population of individual species of storage fungi in parboiled rice showed as in the order of dominance of *A. niger*, *A. candidus*, *A. flavus*, *A. terreus*, Pencillium sp. and *A. glaucus*. The significant and interesting investigation is the presence of less number of Mucor sp. grown in the parboiled grains. The details of percentage occurrence of individual species of fungi in the parboiled raw rice samples are shown in Table 6 and the overall population of fungi is depicted in Graph 4.

TABLE 6. Percentage occurrence of individual species of fungi in Parboiled rice samples

SI.No	*Sample details*	*A.n*	*A. f*	*A. g*	*A.c*	*A.t*	*P. sp.*	*Mucor sp.*	*Total*
1	Pbr 1	13	0	0	31	0	13	0	57
2	Pbr 2	75	0	0	13	0	0	12	100
3	Pbr 3	0	0	0	0	0	0	4	4
4	Pbr 4	86	0	0	4	0	8	0	98
5	Pbr 5	6	33	0	33	0	0	0	72
6	Pbr 6	1	2	2	1	1	0	0	7
7	Pbr 7	1	0	0	0	0	2	0	3
8	Pbr 8	2	0	1	0	2	1	0	6
9	Pbr 9	0	2	0	4	0	0	0	6
10	Pbr 10	0	1	1	0	3	2	0	7
11	Pbr 11	44	33	0	0	0	5	0	82
12	Pbr 12	43	13	0	33	0	10	0	99
13	Pbr 13	26	39	13	10	0	10	0	98
14	Pbr 14	10	0	0	0	70	10	10	100
15	Pbr 15	0	0	0	0	0	0	0	0



GRAPH 4. Percentage occurrence of individual species of fungi in Parboiled rice samples

Analysis of aflatoxins

Out of 10 samples of raw rice samples analysed for the contamination with aflatoxin B_1, 2 samples (sample Rr 1 and sample Rr 7) found with the presence of aflatodxin B_1 in the level of 5 ppm and 3 ppm respectively. Other samples were observed to be negative for the presence of aflatoxin B1. The details of results for the presence of aflatoxin in Raw rice samples shown in Table 7.

For the analysis of 15 Parboiled rice samples for the contamination of aflatoxin B1, it was found that 3 samples (Pbr 5, Pbr 11, Pbr 12) showed positive results for the presence of aflatoxin B_1in the level of 3 ppm, 5 ppm to 8 ppm. The details of results for aflatoxin B_1 analysis in Parboiled rice samples are indicated in Table 8.

Screening of *A. flavus* for aflatoxins

Out of 12 strains of *A.flavus* isolated from raw rice (5)and parboiled rice (7) grains were tested for its aflatoxicigenecity, 8 were positive for production of aflatoxin B_1 in which 3 from raw rice(sample No. 1,7,8) and 5 were from parboiled rice (sample No. 5,6,9,11,13) samples. Overall, the percentage of toxigenic *A.flavus* strains occurred from rice samples was found to be 66.6%.

TABLE 7. Details of Aflatoxin B1 present in Raw rice samples

Sl. No	Samples detailes	Aflatoxin B1 content (ppm)
1.	Raw rice	5
2.	Raw rice	0
3.	Raw rice	0
4.	Raw rice	0
5.	Raw rice	0
6.	Raw rice	0
7.	Raw rice	3
8.	Raw rice	0
9.	Raw rice	0
10.	Raw rice	0

TABLE 8. Details of Aflatoxin B1 present in Parboiled rice samples

Sl. No	Samples detailes	Aflatoxin B1 content (ppm)
1.	Parboiled rice	0
2.	Parboiled rice	0
3.	Parboiled rice	0
4.	Parboiled rice	0
5.	Parboiled rice	3
6.	Parboiled rice	0
7.	Parboiled rice	0
8.	Parboiled rice	0
9.	Parboiled rice	0
10.	Parboiled rice	0
11.	Parboiled rice	5
12.	Parboiled rice	8
13.	Parboiled rice	0
14.	Parboiled rice	0
15.	Parboiled rice	0

DISCUSSION AND CONCLUSION

The present study concludes that the raw rice and parboiled rice samples collected from PDS shops called as ration shop is found mainly used for human consumption by low income group and medium income group people. Since the moisture content of the rice samples were found

under the required norms of rice by from government, it is reflected that the rice is not prone to further contamination with storage fungi during handling and storage. For the anlayis of fungi in the rice samples, the contamination at surface level of rice is found in the normal level which assured that the rice is not spoiled and fit for consumption. However, majority of the rice samples analysed in the present study showed negative results for aflatoxins. Among 15 rice samples analysed for aflatoxins, only 2 samples found with very less amount of aflatoxns which conclude the rice is in safer level.

For the analysis of aflatoxins and food grain spoilage fungi from stored rice variety of PAU 201 from Punjab which were declared unfit for consumption studied by Vasanthi Siruguri, et. al., (2012), and they the results showed that among 35 paddy samples analyzed after milling, more than 90% of samples indicated for the presence of aflatoxin B_1, but none was found to be contaminated with aflatoxins at levels exceeding the prescribed regulatory limits of 30 µg/kg. The results of the study indicated that the stored rice samples did not pose any health concern with respect to aflatoxin contamination as per the criteria laid down by the Food Safety and Standards Authority of India. The above study correlates with our investigation as the rice generally distributed in PDS shops are thought to be lower quality, but the results for the analysis of fungi and aflatoxins showed the rice is safer for human consumption.

The results of the study by Chowdhury *et al.*, 2014 in the rice stored in indigenous storage containers for four months in the laboratory showed the changes of seed such as moisture content, germination, insect and pathogen attack, protein and starch contents over 120 days of storage corroborates the findings of the present study. The results show in case of almost impermeable containers like biscuit tin, drum and hessian bag with polythene cover, seed moisture increased significantly. Moisture percentage of the seeds of biscuit tin, drum and hessian bag with polythene cover was 12.78, 12.95 and 12.88 %, respectively. Germination percentage of seeds in all impermeable containers was above 80 %. Field fungi like *Alternaria, Curvularia, Cladosporium* though observed initially, but in the last pathological test *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. were found predominantly. Insect attack was severe in easily permeable containers. Permeable containers containing the leaves of *neem* and *biskatali* restricted insect and fungal attack. Starch content of seed was 77 % at 60 days of storage and decreased to 75 % at 120 days of storage. The overall observation suggests that, biscuit tin and drum could be used for small scale storage and hessian bag with polythene cover for bulk storage of rice seed for a certain period.

Isolation of food grain spoilage fungi in Libyan food samples including rice with known producer of mycotoxins especially *A. flavus* known to produce aflatoxins, *Aspergillus niger, Aspergillus carbonarious*, *Penicillium chrysogenum* and *Penicillium verrucosum* known to produce ochratoxin and *Fusarium oxysporum* and *Fusarium chlamydosporum* known to produce fumonisins and trichothecenes wich are certainly can pose a health threatening risk for the consumer of those food items and the presence of these fungi in food products could be due to lack of good agriculture and food manufacturing practices throughout the food chain (Alkenz *et al.,*2015). The presence of aflatoxin producing Aspergillus flavus from the rice samples in the present study indicate the above findings and which may health hazard when it has favourable environmental condition in rice during storage. Hence, it is advisable to avoid the contamination of rice with such toxigenic species of fungi through better handling and storage process.

However, large number of samples must be required for arrive definite conclusion to assess the quality of rice samples available from various sources. Rice is the staple food in the state of Tamil Nadu, India. It is marketed in two forms , as raw (untreated rice) and parboiled rice. This study was taken up in order to find the differences between the raw rice and parboiled rice samples with respect to moisture content, mycoflora and presence of aflatoxins. Clearly, rice is a very good natural substrate for the growth of storage fungi. The numbers of micro organisms including fungi in rice. The most commonly occurring storage fungi, however, are a handful of species belonging to the *Aspergillus candidus, flavus, glaucus, niger and terreus* groups and a few species of *penicillium*. These fungi were predent on the surface of all the rice, but had invaded the interior in only about 5-20%. The moisture content (MC) of rice is regarded as a most important factor in fungal deterioration of rice.

Our previous studies show that the population of mesophilic fungi like *A. flavus, A. niger , A.glaucus and terreus* with corresponding decrease in xerophilic ones like *A. glaucus* (Jayaraman and Kalyanasundaram 1989). The parboiled rice acquired a considerable amount of surface mycoflora from the atmosphere. The rice showed a high incidence of *A. flavus* and in one or more samples in lower numbers. A comparative study of the pattern of storage fungi, occurrence of aflatoxins and toxigenic potential of parboiled and raw rice made by studying representative samples of each category indicated that the fungal contamination was higher in raw rice when compared to parboiled rice at low level to moderate level. Owing perhaps to low fungal inoculum, parboiled rice found in laboratory experiments to have greater storability than raw rice, which not only showed an increase in aflatoxin content but became caked with fungal growth after 60 days storage (Jayaraman, 1991; Jayarman and Kalyanasundaram, 1994). Thus the results of contamination of various species of fungi in parboiled and raw rice of the present study indicate the health threatening at condusive environment which can be avoided by using advanced technique of post harvesting methods.

ACKNOWLEDGEMENTS

Authors were acknowledged to Tamil Nadu State Council for Science and Technology (TANSCST)) for providing financial support for the student project (BS-011) in the academic year 2016-17.

REFERENCES

Alkenz, A., Sassi, Y., Abugnah, S. and Alryani, M.B. (2015) Isolation and identification of fungi associated with some Libyan foods. *Afr. J. Fd. Sci.* (7): 406 - 410.

Boesenberg, H. and Eberhardt, E. (1969) Untersuchungen iiber den Verderb von Lebensmittel durch Schimmelpilze in Supermaerkten. *Med. Ernahr* 10:12-13.

Chowdhury, S., Chowdhury, M.A.H., Bhattacherjee, S. and Ghosh, K. (2014) Quality a Storing Grains for Food Security and Sustainability. *Agric. Res*, 1(1):21–24.

Christensen, C.M. (1969) Influence of moisture content, temperature and time of storage upon invasion of rough rice by storage fungi. *Phytopathol. (*59) : 145-148.

Del Prado, F. A., Christensen, C.M. (1952) Grain storage studies. XII. The fungus flora of stored rice seed. *Cereal Chem.* 29: 456-462.

Ellis, M.B. (1971) Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, Pp. 608.

Jayaraman, P. and Kalyanasundaram, I. (1994) Storage mycoflora of parboiled rice through the different stages of processing. *J. Fd. Sci. Technol.* 31, 219-224.

Jayaraman, P. (1991) Storage fungi and aflatoxin B_1 in rice and rice bran, with special reference to parboiling and milling. Ph.D. thesis, University of Madras.
Jayaraman, P (2015)

Jayaraman, P and Indira Kalyanasundaram (1990) Natural occurrence of toxigenic fungi and mycotoxins in rice bran. *Mycopathologia* 110: 81-85.

Jayaraman, P and Indira Kalyanasundaram (2009) Natural occurrence of aflatoxins and toxigenic fungi in rice bran oil and de-oiled bran. Indian J. Sci. Technol. 2(10): 35-36.

Jayaraman, P, Rajesh, V. and Vadamalai Krishnan, K. (2017) Analysis of Formulated Cattle-Feed Samples for the Natural Occurrence of Storage Fungi and Aflatoxin B1. *Int. J. Curr. Microbiol. App. Sci.* 6(2): 1853-1863.

Kurata, H., Udagawa, S.C., Ichinoe, M., Kawasaki, Y., Tazawa, M., Tanabe, H., and Okudaira, M. (1968) Studies on the population of toxigenic fungi in food stuffs. VL Histopathologic changes in mice caused by toxic metabolites of fungi isolated from domestic rice, *J. Food Hyg. Soc. Japan* **9**: 385–394.

Miyake, Y, Sugimara, S and Yasujima, T. (1970) Thorium concentrations and the cativity rations ^{230} Th /^{232}Th and ^{228}Th /^{232}Th in sea water in the western north pacific. *J. Oceanogr. Soc. Japan* 26: 130-136.

Naito, S. (1984) Studies on foliage balight of sugar beets. Res. Bull. Hokkaido Nail. *Agric. Exp. Stn.* 139: 145 – 188.

Onyeze Rosemary, C., Udeh Sylvester, M.C., Akachi Benedict and Ugwu Okechukwu (2013) Isolation And Characterization Of Fungi Associated With The Spoilage Of Corn (*Zea Mays*). *Int. J. Pharma Med. Biol. Sci*., 2(3): 86-91.

Rao, G.J. and Kalyanasundaram, I. (1983) Osmophilism in food grain storage fungi. Mycopathologia. 83: 3-7.

Raper, K.B, and Fennel D.I. (1965) The Genus Aspergillus. The Willium & Wilkins Company, Baltimore. Pp. 686.

Raper, K.B, Thom, C. and Fenne, D.I. (1949) A manual of the Penicillia. The William & Wilkins Company, Baltimore, Pp. 878.

Reddy, K. R. N., Reddy, C.S., Abbas, H. K., Abel, C. A. And Muralidharan, K. (2008) Mycotoxigenic Fungi, Mycotoxins, And Management Of Rice Grains', Toxin Rev. 27(3): 287-317.

Udagawa, S. (1959) Taxonomix studies of fungi on stored rice grains. Penicillium group. *J. Agri. Sci. Tokyo Daigaku.*5: 5-21.

Uma, V and Wesely, E.G. (2013) Seed borne fungi of rice from South Tamil Nadu. *J. Acad. Indus. Res*, 1(10): 612-614.

Vasanthi Siruguri, P Uday Kumar, P. Raghu, M Vishnu Vardhana Rao, B. Sesikeran, G. S. Toteja, Priyanka Gupta, Spriha Rao, K. Satyanarayana, V.M. Katoch, T.S. Bharaj, G.S. Mangat, Neerja Sharma, J.S. Sandhu, V.K. Bhargav, and Shobha Ran (2012) Aflatoxin contamination in stored rice variety PAU 201 collected from Punjab, India, Indian J Med Res. 136(1): 89–97.

Wajiha Iram, Tehmina Anjum, Rasheda Jabeen and Mateen Abbas. (2018) Isolation of Stored Maize Mycoflora, Identification of Aflatoxigenic Fungi and its Inhibition using Medicinal Plant Extracts, *Int. j. Agri. Biol.*, (20): 2149–2160.

Tsuruta, O. (1973) Storage of husked rice in cave: Changes in mycoflora of husked rice. *Rep. Natl. Fd. Res. Inst.* 28: 10-18.

Tsuruta, O and Saito, M. (1980) Mycological damage of domestic rice during storage in warehouses under natural conditions: Changes in mucoflora during the storage. *Tran. Mycol. Soc. Japan* 21: 121-125.

Shiela V, Vijaya, K and Kalyanasundaram, I. (1978) Fungal spilage of stored foodgrains – A preliminary sudy with rice . *J. Madras Univ. (Sec. B)*, 41: 149-160.

Seitz L.M and Mohr, H.E. (1974) Aflatoxin detection in corn: A simple screening test. *Cereal Chem*. 51: 487- 491.
Bullerman L.B. (1974) A screening medium and method to detect several mycotoxins in mold cultures. *J. Milk FD. Technol*. 37: 1-3.