EFFECT OF STORAGE ON SEED BORNE AFLATOXIN PRODUCING MYCOFLORA IN WHEAT, PADDY AND RICE

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Abstract- Wheat, paddy and rice are widely cultivated cereal crops in India. In the present study, the stored samples of wheat, paddy and rice were collected from SWC (State Warehousing Corporation) Rampura Phul, CWC (Central Warehousing Corporation) Moga and FSD (Food Supply Depot) Khanna under covered storage, FSD Moga under cover and plinth storage. The storage period was 18 months. The samples were analysed after every three months, for the total incidence of mycoflora and aflatoxin contamination. The seed associated mycoflora was isolated by using standard blotter paper method. Percentage (%) incidence of seed borne fungi increased during the entire storage. *Asergillus* sp. was recorded at maximum level, followed by *Rhizopus* sp. and *Penicillium* sp. Aflatoxins were analysed qualitatively and quantitatively by using Pressure Mini Column method and Liquid Chromatographic method, respectively. The incidence of aflatoxin contamination increased during the storage. Aflatoxin contamination was found Below Detection Limit (Method Detection Limit = 5µg/kg) for all the samples, which showed positive results for aflatoxin contamination during qualitative assessment. The incidence of seed borne mycoflora and aflatoxin contamination was higher in Khanna, followed by that in Moga and Rampura Phul. The samples taken from CAP storage had higher incidence of mycoflora and aflatoxin contamination as compared to that in the covered storage. Hence, covered storage was better for maintaining the microbiological quality.

Keywords: **Stored grains, Seed borne mycoflora, Aflatoxin, Storage conditions.**

INTRODUCTION

India is a world leader in the production of food grains. The total production of food grains in India is 251.27 million tons (Anonymous 2016). The most favoured grains are wheat, rice, corn, sorghum, barley, millet, rye, oats and paddy. Grain storage plays a major role in the economy of developed and developing world (Ellis *et al* 1992). The grains are stored with government agencies such as: Central warehousing Corporation (CWC), State Warehousing Corporation (SWC) and Food Corporation of India (FCI). The most widely used storage methods are Cover and Plinth storage (CAP) and covered storage, which are inexpensive but loss of grains is unavoidable. The grain quality in storage depends on major factors such as: environmental conditions during the period of storage, initial condition of the grain and biotic factors like rodents, insects and micro-organisms. Moisture content is one of the key factors determining the storability of grains. Fungi present a more serious problem for cereals. Being more tolerant to reduced water activity than bacteria, fungi can easily grow and leads to spoilage in various cereal commodities. The field fungi attack the grains developing on the plants in the field or after the seeds have matured and plants are still standing and awaited. While, storage fungi involved in the deterioration of grains during storage right after threshing. They all have the capability to grow in materials, whose moisture content is in equilibrium with relative humidity of 60-90%.

Fungal growth is a significant problem during the storage period comprising the potential production of mycotoxins and also induces decrease in germinability and nutritional value (Scudamore 1993). Mycotoxins are secondary metabolites of fungi, which are able to cause chronic or acute toxic effects on humans and animals. Aflatoxins are widely known mycotoxin contaminants. These are produced by *Aspergillus flavus* and *A. parasiticus* (Oliveira *et al* 2009). There are four most common types of aflatoxins: aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2. Among them, aflatoxin B_1 is the most prevalent, toxic and as well as the most hazardous for its ability to induce liver cancer in humans. Aflatoxins commonly occur in corn, groundnuts, wheat, rice, cottonseed, copra, milk, cheese and eggs. By taking account of all these effects it is essential to estimate fungi associated with cereals and control of microbial toxins (aflatoxins) in food is one of the most pressing food safety issues confronting the food industry.

MATERIALS AND METHODS

Collection of grain samples and their storage

Wheat samples were collected from FSD (Food supply Depot) Moga under cover and plinth (CAP) storage, CWC (Central Warehousing Corporation) Moga and SWC (State Warehousing Corporation) Rampura Phul, under covered storage. Paddy samples were collected from the FSD Moga under CAP storage. Samples of rice were collected from FSD khanna, CWC Moga and SWC Rampura Phul, under covered storage (Table 1).

Grain samples from the stacks were collected as per the standard sampling technique i.e. either M shaped or W shaped (Anonymous 1978). Grain samples were collected from all the five sides of the stack that can represent whole of the stack. Samples were collected after every three months. Grain samples of wheat, paddy and rice were stored for 18 months (February

2015-August 2016).

Table 1: Locations and sources of grain samples of different cereals

Sampling Location	FSD, Khanna	SWC, Rampura phul	CWC, Moga	FSD, Moga	Total samples
Storage type	Covered storage	Covered storage	Covered storage	CAP storage	
Seed source/ Category	Ch-1 (Rice) Ch-2 (Rice)	Ch-54 (Wheat) Ch-55 (Wheat) Ch-53 (Rice) Ch-56 (Rice)	Ch-33B (Wheat) Ch-35B (Wheat) Ch-16B (Rice) Ch-16C (Rice)	Ch-OP6 (Wheat) Ch-OP (Paddy)	
Rice	2	2	2	-	6
Wheat	-	2	2	1	5
Paddy	-	-	-	1	1
Total samples	2	4	4	2	12

Ch-chamber; SWC- State Warehousing Corporation; CWC- Central warehousing Corporation; FSD- Food Supply Depot

Isolation of mycoflora

The mycoflora of the samples under Cover and Plinth storage (CAP) and covered storage were isolated by the blotter method (International Seed Testing Association 1976). Surface sterilization of the samples was carried out by 0.1 % mercuric chloride (Bhutta 1988, Arora *et al* 1994). Half of the seeds were pre-treated with 0.1 % mercuric chloride and other half were untreated. The three layers of blotters (size equivalent to the size of petridish) were jointly soaked in distilled water and kept in petridish. Surplus water was drained from the blotters. Ten seeds were placed at a equal distance in the petridish. Ten petridishes were used for one sample (100 seeds). After placing the seeds, the petridishes were incubated for 7 days at 25 ± 2 ºC.

The seeds were examined on 8th day under stereoscopic microscope. The identification criteria were based on the basis of sporulation (arrangement of conidia with conidiophores) and their fruiting bodies etc. (Alexopoulos *et al* 1996).

Qualitative analysis of aflatoxins from the samples of wheat, paddy and rice

Mycotoxins are secondary fungal metabolites and produced by some phytopathogenic spoilage fungi such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alterneria* species. Aflatoxins are the most prevalent mycotoxins. The four main naturally-occurring aflatoxins are aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2. Among them, aflatoxin B_1 is the most prevalent, toxic and the most dangerous, for its ability to cause liver cancer in humans. Aflatoxins were analysed qualitatively by using Pressure Mini Column method (Sashidhar *et al* 1989). The method followed was extraction of aflatoxins, activation of pressure mini column and detection by fluorescence.

Preparation of Column: A column was taken and a filter paper disc was placed at the bottom, then a layer of anhydrous sodium sulphate (3 mm) was added. Then, a layer of silica gel (5 mm) was made above this layer and then florisil (1 mm) was added and again filter paper was inserted. Then again, a layer of sodium sulphate (4 mm) was added and column was closed by placing a filter paper disc. After making the column, it was placed in oven till the temperature reached 110 ºC. Then, the column was removed from oven and allowed to cool.

Procedure: Samples (10 g) were taken in a flask and 50 ml of acetone water solution (Acetone: Water; 85:15) was added and then the samples were kept on a rotary shaker for one hour. After rotation, samples were filtered through Whatman filter paper No.1. To 10 ml of the filtrate, 10 ml of 20 % of lead acetate solution was added and mixed thoroughly and again filtered through the filter paper. To the filtrate, 2 ml of benzene was added and mixed thoroughly for 3-4 min. Upper benzene layer was collected in a test tube containing 400 mg of neutral alumina. The sample (1 ml) was then loaded in the column and 3 ml of clearing solvent (hexane: chloroform: tetrahydrofuran in 70:20:10) was added and pressure was applied with the piston to drain the solvent. The samples were then analysed under UV light chamber at 365 nm for the presence of compact blue fluorescence band at the interface of florisil and silica gel.

Quantitative assessment of aflatoxins from the samples of wheat, rice and paddy

Several methods have been developed for detection and quantification of aflatoxins from agricultural produce. These methods include Liquid Chromatographic method, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Fluorescence Spectrophotometry and Enzyme Linked Immunosorbent Assay (ELISA). The quantitative assessment of the positive samples were analysed by using Liquid Chromatographic method (AOAC 990.33). The method followed was extraction and purification of aflatoxins, followed by derivatization with tri-fluroacetic acid, separation by using reverse-phase liquid chromatography method and detection by fluorescence. The method detection limit was 5 µg/kg.

Statistical analysis

Data was collected in triplicate and analysis of variance (ANOVA) technique was used to analyse the data and to compare the mean difference of samples. The statistically difference was defined as $p < 0.05$. Standard error was calculated manually for all the experiments.

RESULTS AND DISCUSSION

Isolation and identification of fungal species

Mycoflora contamination of the stored grains are influenced by the type of cereal host, storage conditions and duration. Mycoflora was isolated from various grains through standard blotter paper method (International Seed Testing Association 1976).

The colony colour and microscopic properties such as spore size and shape of each isolate were used for identification of fungi up to genera level by comparing with synoptic key (Alexopoulos *et al* 1996). On the basis of these microscopic and morphological characteristics, three genera of fungi were identified in wheat samples and two genera of fungi were found in paddy and rice samples.

Genera of fungi isolated from stored wheat grains

The prominent seed associated mycoflora of wheat during the storage of 18 months (February 2015 – August 2016) was recorded and analysed at three months interval. The fungi isolated from wheat grains are depicted in Table 2. In case of wheat grains, three genera of fungi were isolated. They include *Aspergillus* sp., *Rhizopus* sp. and *Penicillium* sp. *Rhizopus* sp. and *Aspergillus* sp. were observed in all the tested wheat grain samples. *Aspergillus* sp. was recorded at the maximum level (51.2-76.6 %), followed by *Rhizopus* sp. (2-6 %) and *Penicillium* sp. (0.5-2.0 %). During the storage of wheat grains, the storage period significantly ($P < 0.05$) affected the mycoflora of wheat grains. It was found that fungal contamination increased significantly ($P < 0.05$), over the storage period up to 18 months. It was mainly due to an increase in the moisture content of the grains during storage.

The results shown in Table 2 revealed that during storage, different locations significantly ($P < 0.05$) affected the mycoflora of wheat grains. Samples from Rampura Phul had the least contamination, while the samples from Moga were associated with maximum contamination. Percentage (%) incidence of seed borne fungi associated with wheat grains ranged from 59.1-84.6 % in CAP storage and lesser in covered storage (51.2-78.6 %). Data showed that in all the different locations during storage, the wheat grains which were pre-treated with mercuric chloride showed lesser percentage (%) incidence of seed borne fungi than untreated grains.

Genera of fungi isolated from stored paddy and rice grains

The results depicted in Table 3 show that only two genera of fungi were isolated from paddy and rice samples. They include *Aspergillus* sp. and *Rhizopus* sp. *Aspergillus* sp. recorded a maximum level of occurrence (40.9-65 %), followed by *Rhizopus* sp. (2.1-10.1 %). It was found that the fungal contamination associated with rice and paddy grains increased significantly ($P < 0.05$), during the storage period of 18 months.

Similarly, various locations significantly ($P < 0.05$) affected the mycoflora of rice and paddy grains, during the storage. Among three different locations, samples from Rampura Phul had the least contamination of seed borne mycoflora, followed by that in Moga and Khanna. Percentage (%) incidence of seed borne fungi ranged from 49.1-72.6 % in CAP storage and lesser in covered storage (43.0-70.2 %). Data showed that in all the different locations during storage, rice and paddy grains which were pre-treated with mercuric chloride showed lesser percentage (%) incidence of seed borne fungi than untreated grains.

El-Kady *et al* (1982) reported that *Aspergillus* sp., *Rhizopus* sp. and *Penicillium* sp. were the most frequently isolated genera in the cereal samples analyzed. It was stated that *Aspergillus* sp. is the most common fungal contamination on stored rice seeds for more than a year from 18 different ecosystems (Reddy *et al* 2004). Katta and Bullerman (1995) observed that fungal contamination increased with increase in time. They concluded that manipulation of abiotic factors like moisture content and temperature of the corn seeds have a significant impact on seed borne fungi. Percent frequency of seed borne mycoflora isolated from maize samples, increased with increase in storage period (Hell *et al* 2000). Kandhare (2016) observed that seed borne mycoflora associated with green gram increased during the storage period of 12 months.

Reed *et al* (2007) found that the higher initial moisture content leads to increase in infection of maize kernels, while studying the impact of temperature and moisture content on storage moulds. Chauhan *et al* (2008) demonstrated that higher temperature favours the growth of *Aspergillus* species. Chulze (2010) reported the influence of storage environment on the seed mycoflora of crops. During storage, the grain temperature was higher in CAP storage as compared to the covered storage. In CAP storage, it increased from 29.10-39.94 °C and in covered storage it increased from 29.30-32.31 °C. It was due to the heat released by micro-organisms and insects during respiration (Sawant *et al* 2012). Surface sterilization of wheat and paddy samples with 0.1 % mercuric chloride showed lesser frequency (%) of occurrence of fungi as compared to that of untreated grains (Arora *et al* 1994).

Table 2: Percentage (%) incidence of seed borne fungi in wheat

Storage period (months)	Prevalence of seed borne fungi (%) in wheat samples																																							
	Covered storage																																		Cover and plinth storage (CAP)						
	RWC 54							RWC 55							MWC 33B							MWC 35B							MW CAP OP6						
	R		A		P		ST	R		A		P		ST	R		A		P		ST	R		A		P		ST	R		A		P		ST
	NT	T	NT	T	NT	T		NT	T	NT	T	NT	T		NT	T	NT	T	NT	T		NT	T	NT	T	NT	T		NT	T	NT	T	NT	T	
0	2.0	-	48.7	-	0.5	-	51.2	2.1	-	49.0	-	0.5	-	51.6	2.5	-	51.9	-	0.5	-	54.9	2.6	-	52.0	-	0.5		55.1	3.2	-	55.4	-	0.5	-	59.1
3	2.3	-	52.0	-	0.5	-	54.8	2.3	-	51.5	-	0.5	-	54.3	2.9	-	57.5	-	0.5	-	60.9	3.0	-	55.7	-	0.5	-	59.2	3.3	-	60.3	1.0	1.0	-	64.6
6	2.8	-	7.6	0.5	0.5	-	60.9	2.9	-	56.8	0.5	0.5	-	60.2	3.2	-	61.0	1.0	0.5	-	64.7	3.4	-	59.1	0.5	0.5	-	63.0	3.5	-	63.0	1.2	1.0	-	67.5
9	3.2	-	63.5	0.5	1.0	-	67.7	3.1	-	62.3	0.5	1.0	-	66.4	3.5	-	65.6	1.0	1.0	-	70.1	3.5	-	64.4	1.0	1.0	-	68.9	3.8	-	71.0	1.0	1.0	-	75.8
12	3.3	-	65.1	1.0	1.0	-	69.4	3.3	-	65.0	0.5	1.0	-	69.3	4.0	-	68.2	1.0	1.0	-	73.2	4.0	-	68.0	1.0	1.0	-	730	4.6	-	71.4	1.5	1.0	-	77.0
15	3.8	-	68.3	-	1.0	-	73.1	3.5	-	67.3	1.0	1.0	-	71.8	4.4	-	70.3	1.5	1.0	-	75.7	4.6	-	70.6	1.0	1.5	-	76.7	5.5	-	74.3	1.5	1.5	-	81.3
18	4.4	-	69.2	1.5	1.5	-	75.1	4.3	-	68.6	1.0	1.5	-	74.4	4.8	-	71.9	2.0	1.5	-	78.2	4.8	-	72.3	1.5	1.5	-	78.6	6.0	-	76.6	2.0	2.0	-	84.6

CD (5%)

A : 0.22
B : 0.19
A*B : 0.50
C : 0.14
A*C : 0.39
B*C : 0.33
A*B*C : 0.88

Average of three replicates

RWC- Rampura Phul Wheat Covered; MWC- Moga Wheat Covered; MW CAP- Moga Wheat CAP; A- *Aspergillus*; R- *Rhizopus*; P- *Penicillium*

Storage period- 18 months; T- Treated with 0.1% mercuric chloride; NT- Not treated; ST- Sum total

Table 3: Percentage (%) incidence of seed borne fungi in paddy and rice

Storage period (months)	Prevalence of seed borne fungi (%) in rice samples																																					
	Covered storage																																		Cover and Plinth storage (CAP)					
	RRC 53					RRC 56					MRC 16B					MRC 16C					KH-1					KH-2					MP OP									
	A		R		ST	A		R		ST	A		R		ST	A		R		ST	A		R		ST	A		R		ST	A		R		ST				
	NT	T	NT	T		NT	T	NT	T		NT	T	NT	T		NT	T	NT	T		NT	T	NT	T		NT	T	NT	T		NT	T	NT	T				
0	40.9	-	2.1	-	43.0	41.0	-	2.5	-	43.5	42.6	-	2.9	-	45.5	41.8	-	3.0	-	44.8	42.8	-	5.1	-	47.9	42.6	-	4.8	-	47.4	45.6	-	3.5	-	49.1				
3	42.8	-	3.0	-	45.8	42.3	-	3.0	-	45.3	44.5	0.5	3.2	-	47.7	44.1	0.5	3.2	-	47.3	47.0	-	5.8	-	52.8	47.6	-	5.5	-	53.1	52.5	-	4.2	-	56.7				
6	43.0	-	3.5	-	46.5	43.5	-	3.3	-	46.8	46.9	1.0	3.4	-	50.3	45.8	0.5	3.5	-	49.3	48.7	1.0	6.3	-	55.0	49.9	1.0	6.7	-	56.6	57.9	1.0	4.9	-	62.8				
9	45.1	0.5	3.8	-	48.9	44.8	0.5	3.3	-	48.1	49.0	1.0	4.1	-	53.1	48.5	1.0	4.3	-	52.8	51.9	1.0	7.0	-	58.9	53.0	1.5	7.4	-	60.4	60.0	1.0	5.5	-	65.5				
12	47.6	0.5	4.0	-	51.6	47.0	0.5	3.5	-	50.5	53.3	1.0	4.7	-	58.0	52.1	1.0	4.8	-	56.9	57.8	1.0	8.2	-	66.0	57.2	1.5	8.9	-	66.1	61.2	1.0	6.2	-	67.4				
15	49.8	0.5	4.6	-	54.4	48.2	1.0	4.5	-	52.7	56.5	1.5	5.5	-	62.0	54.0	1.0	5.4	-	59.4	59.7	1.5	9.7	-	69.4	58.5	1.5	9.6	-	68.1	63.5	1.5	7.0	-	70.5				
18	51.7	1.0	4.7	-	56.4	50.1	1.0	4.6	-	54.7	58.5	1.5	5.8	-	64.3	58.2	1.0	5.9	-	64.1	60.3	1.5	9.9	-	70.2	60.0	1.5	10.1	-	70.1	65.0	1.5	7.6	-	72.6				

CD (5%)

A : 0.18
B : 0.18
A*B : 0.49
C : 0.10
A*C : 0.26
B*C : 0.26
A*B*C : 0.70

Average of three replicates

RRC- Rampura Phul Rice Covered; MRC- Moga Rice Covered; KH- Khanna Rice; MP CAP- Moga Paddy CAP; A- *Aspergillus*; R- *Rhizopus*;

Storage period- 18 months; T- Treated with 0.1% mercuric chloride; NT- Not treated; ST- Sum total

4.3 Detection of aflatoxins in the samples

The qualitative as well as quantitative detection of aflatoxins in the samples is shown in Table 4-5 and Plate 1. It was found that incidence of aflatoxin contamination in wheat, paddy and rice samples increased with increase in storage time. The wheat samples from Rampura Phul did not show any positive result for aflatoxin contamination under covered storage. Aflatoxin contamination in Moga (covered storage) wheat samples was detected after 14 months of storage, while in Moga (CAP storage), aflatoxin contamination was detected during 12-18 months of storage. Similarly, paddy and rice samples from Rampura Phul did not show positive results for aflatoxin contamination under covered storage. Aflatoxin contamination in Khanna (covered storage) rice samples was detected during 15-18 months of storage. Aflatoxin contamination in Moga (covered storage) rice samples was detected during 18 month of storage, while in Moga (CAP storage) paddy samples aflatoxin contamination was detected during 9-18 months of storage.

Aflatoxin contamination was found Below Detection Limit (Method Detection Limit = 5 µg/Kg) for all the samples, which showed positive results for aflatoxin contamination during qualitative assessment. Aflatoxin analysis of samples indicated that none exceeded the tolerance limit of 30 µg/kg (Food Safety and Standards Regulations 2011). In the present study, the extent of aflatoxin contamination found in the stored samples was consistent with reported studies on aflatoxin contamination in rice, with majority of samples having levels below the tolerance limits for aflatoxin in India and abroad (Vasanthi and Bhat 1990, European Union 2003, Reddy *et al* 2009). The risk of aflatoxin contamination increased in agricultural commodities for long periods of storage. The content of important nutrients can also be reduced (Toth *et al* 2013).

Table 4: Aflatoxin contamination in wheat

Storage period (months)	Aflatoxin contamination in wheat samples				
	Covered storage				Cover and plinth storage (CAP)
	RWC 54	RWC 55	MWC 33B	MWC 35B	MW CAP OP6
0	-	-	-	-	-
3	-	-	-	-	-
6	-	-	-	-	-
9	-	-	-	-	-
12	-	-	-	-	+ (BDL)
15	-	-	+ (BDL)	-	+ (BDL)
18	-	-	+ (BDL)	+ (BDL)	+ (BDL)

(-) = absent; BDL-Below Detection Limit (MDL= Method Detection Limit=5µg/Kg)

Average of three replicates

RWC- Rampura Phul Wheat Covered; MWC- Moga Wheat Covered; MW CAP- Moga Wheat

CAP Storage period- 18 months

Table 5: Aflatoxin contamination in rice and paddy samples

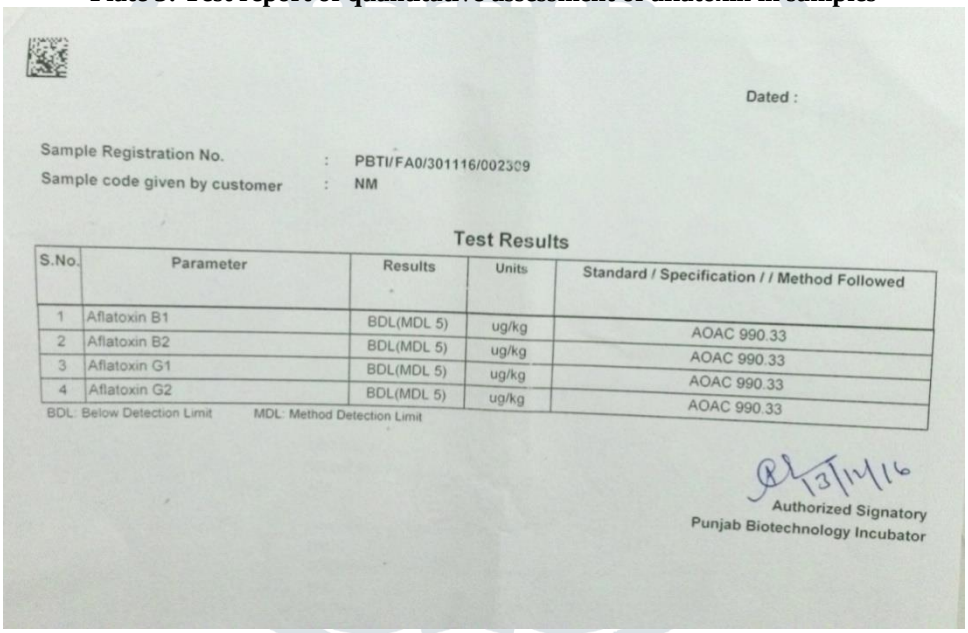
Storage period (months)	Aflatoxin contamination in rice samples						
	Covered storage						Cover and Plinth storage (CAP)
	RRC 53	RRC 56	MRC 16B	MRC 16C	KH-1	KH-2	MP OP
0	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
9	-	-	-	-	-	-	+ (BDL)
12	-	-	-	-	-	-	+ (BDL)
15	-	-	-	-	+ (BDL)	+ (BDL)	+ (BDL)
18	-	-	-	+ (BDL)	+ (BDL)	+ (BDL)	+ (BDL)

(-) = absent; BDL-Below Detection Limit (MDL= Method Detection Limit = 5µg/Kg)

Average of three replicates

RRC- Rampura Phul Rice Covered; MRC- Moga Rice Covered; KH- Khanna Rice; MP CAP- Moga Paddy CAP; Storage period- 18 months

Plate 3: Test report of quantitative assessment of aflatoxin in samples



Dated :

Sample Registration No. : PBTI/FA0/301116/002309
Sample code given by customer : NM

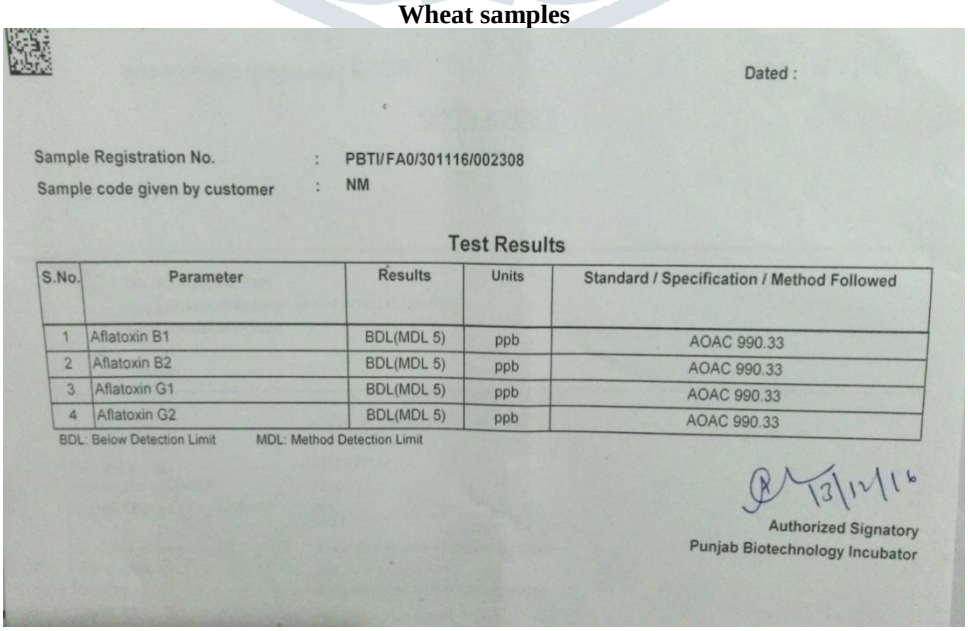
Test Results

S.No.	Parameter	Results	Units	Standard / Specification / / Method Followed
1	Aflatoxin B1	BDL(MDL 5)	ug/kg	AOAC 990.33
2	Aflatoxin B2	BDL(MDL 5)	ug/kg	AOAC 990.33
3	Aflatoxin G1	BDL(MDL 5)	ug/kg	AOAC 990.33
4	Aflatoxin G2	BDL(MDL 5)	ug/kg	AOAC 990.33

BDL: Below Detection Limit MDL: Method Detection Limit

13/12/16
Authorized Signatory
Punjab Biotechnology Incubator

Wheat samples



Dated :

Sample Registration No. : PBTI/FA0/301116/002308
Sample code given by customer : NM

Test Results

S.No.	Parameter	Results	Units	Standard / Specification / Method Followed
1	Aflatoxin B1	BDL(MDL 5)	ppb	AOAC 990.33
2	Aflatoxin B2	BDL(MDL 5)	ppb	AOAC 990.33
3	Aflatoxin G1	BDL(MDL 5)	ppb	AOAC 990.33
4	Aflatoxin G2	BDL(MDL 5)	ppb	AOAC 990.33

BDL: Below Detection Limit MDL: Method Detection Limit

13/12/16
Authorized Signatory
Punjab Biotechnology Incubator

Rice samples

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