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Deterioration in Quality and Quantity of Wheat Grains during Storage

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Abstract: ***Food is the basic requirement of life and hence need more attention before its consumption. Approximately one fifth of the total agricultural produce either does not reach to consumer or become unsafe for use. However, the most unsafe portion of food grains usually falls to the lot of the poorer. Proper agronomic care of the crops and adequate precautions at the time of harvesting and storage can be of substantial help in avoiding the risk. The former aims at ensuring best kind of propagations materials and maximum production of food grains, while the letter is devoted to conservation and utilization of human food in its best form. However, the quality of food grains is of great importance. Any change in the property of seed, therefore, has to be thoroughly examined as to whether it is in accordance with the norms of food for which the grains are meant. In the present investigation, deterioration in quality and quantity of wheat seed/grains, were analyzed.***

Keywords: Deterioration, quality loss, stored grain, wheat, fungal infestation.

1. Introduction

Fungi are one of the major factors restricting the storability of seed grains. Accordingly, prevention of grain deterioration by fungal contaminants is the most important task relating to all systems for storing the food grains after harvesting. The post harvest management or store management depends on the satisfactory manipulations of biotic and abiotic factors to minimize grain deterioration during storage (Lacey, 1989).Cereal grains are rich sources of nutrients, not only for succession of fungi, and other micro-organisms but also for insects and mites, whose growth decrease its value as human and animal food (Sinha, 1973; Lacey, 1989).The time of harvesting, the method of thracing and the environmental conditions at the farm during thracing period have definite influence on the type of contaminants in the seed grain. Another major factor which is considered to be important for subsequent development of micro-organism on grains during storage is the how and when the grain is transported from farm to the storages. Flannigan (1978) also reported that the grains are exposed to a high risk of contamination especially with species of Aspergillus and Penicillium during their transportation from field to storages.

Literally speaking, storage fungi are those that grow on storage products. Some of them are able to grow on substrates without free water, and on media with a higher osmotic pressure. Most of the storage flora includes species of Aspergillus and Penicillium which are active at relative humidity's ranging from 70-90 percent. It has been established that the storage fungi usually do not invade grain before harvest (Christensen and Kaufmann, 1969; Christensen, 1971) but they may be found on the seed in very low percentage and serve as inoculums of storage fungi (Tuite, 1959,1961; Qasen and Christensen, 1958). They may be present as surface contaminants and may also be present as dormant mycelium within the tissues of pericarp or seed coat (Warnock and Preece, 1971). To determine the specificity of fungal flora, moisture content of grain is one of the most important factors and a slight variation in the moisture content may alter the composition of fungal population both qualitatively and quantitatively. The predominant species of storage fungi belongs to genus Aspergillus of which Aspergillus restrictus, A.amstelodemi, A.repens and A.ruber are most common (Christensen and Kaufmann, 1969). In addition to these, Willing (1974) found Penicillium cyclopium as a dominant fungus in Danish stored barley grain. Christensen and Lopez (1963) reported the invasion of A. holophilicus and A. restrictus at 15.0 %, A. ochraceous at 15.2% and A. flavus at 18.0% water content of wheat, barley, maize and sorghum grains. He also reported that an increase of 0.2% moisture content may lead to many fold increase in number of colony forming units (cfu) of A. restrictus in wheat grain stored at 25°C temperature for 100 days. Literature showed that biochemical mechanisms operative in saprophytic seed inter-relationship function efficiently and systematically in favor of the fungus. Thus the general supposition has been that the saprophytic fungi simply degrade organic substrate to yield compounds which will be more readily available to fulfill their nutritional and metabolic requirements. But studies involving the changes in nutritional qualities of infested cereal grains relative to human needs have received only meager attention.

Thermophilous fungi are capable of deteriorating cereal grains at temperature where mesophilic fungi generally fail to grow. The investigations made so for on this important aspects of thermophilous fungi are not sufficient to characterize the loss of grains in quality and quantity in comparison to other mesophilic fungi which have been thoroughly investigated (Christensen, 1978; Corry, 1978; Mills and Wallace, 1979). In view of the large scale deterioration of grains under Indian conditions, present study is proposed to characterize the role of thermophilous fungi in the deterioration of wheat grain during storage. Under deterioration studies the change in free amino acids, reducing sugars and oils yield, were studied. The type of changes occurring in seed grain closely related to the pattern, how a mould is entering in the seed i.e., through the pericarp or through the germ is an equally important

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aspect which require a systematic study. In view of this, our present study was planned to evaluate the loss in germinability of seed grain and loss in fiber content. Lipolytic activity of fungi may suggest their possible role in the deterioration of wheat germ and hence an attempt is also made to study extracellular production of various lipolytic enzymes by test fungi. The studies are also performed to investigate that which group of fungi are more damaging, whether mesophilic or thermophilic, when they were provided suitable temperature in the laboratory condition for storage of wheat grains.

2. Materials and Methods

Present study deals with the quality loss in wheat grains having different water content during their storage at mesophilic and thermophilic temperature due to fungal infestation. The details of materials and methods are as follow:

2.1 Test organisms: Selection of test organisms was done on the basis of the data obtained in the survey of fungal occurrence in wheat grain under various storages. A total of four test fungi, which includes two strains of thermophilic Aspergillus nidulans TH24 (IMI No 321214) and Aspergillus niger TH147 (IMI No. 315151), and two strains of mesophilic i.e. Emericella nidulans M 38 (IMI No.325152) and Aspergillus flavus (IMI No. 325149) were selected for present study. These were of common occurrence in the samples of wheat grain of the surveyed area of Madhya Pradesh and Chhattisgarh state of India.

2.2 Preparation of grains having different water content: Grains of wheat var. WH 147 without any dressings were obtained from Madhya Pradesh Rajya Beej Evam Farm Vikash Nigam, Sagar and used in the present investigation. Grains were wetted to 12.0, 15.0 18.0 and 21.0 percent water contained (WC). After equaliberation over a period of 5-6 days at 4°C±2°C with frequent mixing, 100 grams amounts were placed in sterilized 250 ml Erlenmeyer flasks. Approximate 100 g unwetted grain of wheat (with having 9.99% WC), were also placed in flasks and were run as control (grain as such).

2.3 Inoculation of grain: Spore of thermophilous Aspergillus nidulans TH24 (IMI No 321214) and Aspergillus niger TH147 (IMI No. 315151), and two strains of mesophilic i.e. Emericella nidulans M 38 (IMI No.325152) and Aspergillus flavus (IMI No. 325149), were harvested in 0.1% Tween-80 from seven days old fungal colonies grown on malt salt agar (2%), and spore suspension was standardized at $2x10^6$ spore/ml. Erlenmeyer flasks containing grain were then inoculated with 1 ml of spore suspension and mixed well by shaking. A set of wetted grains of each water contents, were left uninoculated and served as control II.

2.4 Storage of grain: Grains inoculated with thermophilic strains i.e., A. niger (TH 147) and A. nidulans (TH 24) and with mesophilic strains i.e., A. flavus (TH61) and E. nidulans (M38) were stored at 45°C±1°C and 28°C±1°C, respectively, along with controls. To minimize loss of water from grains at 45°C, relative humidity equivalent to 80% was maintained in the incubator. Required amount of grains were taken out from flasks as per study plan and were processed further to study the following:

2.4.1 Grain Mycoflora: Wheat (9.99% WC) grains were placed on two media i.e., Malt extract agar and Malt salt agar (2%) and incubated at 45°C±1°C and 28°C±1°C to study the fungal contaminants in them in the beginning of the experiment. Fungi appearing on grain were recorded after 3 days of incubation. Seed health was tested by sowing test grains on moistened filter paper pad in Petri dishes and percentage of germinating seeds was recorded after 3 days (Shukla, 1991).

2.4.2 Changes in free amino acids composition during molding of wheat grain: For extraction of free amino acids and sugars, 5 grams of defatted powdered grain were extracted with 80% ethyl alcohol and then with water (Mahadevan and Sridhar, 1983). The two extracts (i.e., alcoholic and water extracts) were combined and condensed by evaporation to semi dryness. The dried extract was then dissolved in 80% ethanol and centrifuged at 3000 rpm for 15 minutes to obtain clear extract. So obtained grain extracts were then examined for free amino acid and sugars analysis by chromatographic methods. An amount of 25 µl of the extract were loaded on chromatographic grade paper, Whatman No. 1 with the help of 25 µl micropippettes with intermittent drying, and were run in the solvent system containing n-butanol: acetic acid: water (12:3:5). After about 80% length of paper covered by the solvent run, the paper was then removed, dried at room temperature and then sprayed by ninhydrin solution (300 mg of commercial ninhydrin dissolved in 100 ml of ethyl alcohol). After spray the paper was dried at 105°C±2°C in chromatographic oven and developed spots were identified by comparing the R_f -values of the spots with the known standard. Intensity of the colored spots was recorded on an arbitrary scale by visual observations. The data given in table 2&3 represents an amount of amino acids in grain samples as recorded after 60 days incubation and the order of their appearance after 15, 30 and 60 days incubation is given in parenthesis against each datum (Shukla, 1991).

2.4.3 Change in sugar content of wheat grains during molding: The grain extracts were analyzed for sugars by thin layer chromatography (Stahl, 1965). An amount of 25 µl extracts were loaded on plates. For separation of sugars a solvent system containing n-butanol : Acetic acid : water (4:1:5) was used for the study. After running the solvent the plates were then dried and sprayed with Aniline hydrogen phthate reagent (Aniline reagent was prepared by dissolving Aniline 9.2 ml, phthalic acid 16 grams in mixture of n-butanol 492 ml, diethyliether 490 ml and 20 ml of distilled water). After drying, the spots were identified with the help of known standard sugars. Amount of sugars as indicated by intensity of colored spots was recorded an arbitrary scale. Amount of sugars are denoted by numerical numbers, while the order of decrease, increase or otherwise as examined after 15, 30 and 60 days is given in parenthesis in the tables 4.

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2.4.4 Change in reducing sugar content of wheat grains: Extraction of sugars from grain was using the method as described by Mahadevan and Sridhar, (1983). For this, 5 grams of grains were made fat free by ether extraction and then ether was removed by air drying. The grains were blended and placed in 100 ml beakers containing 25 ml of 80% alcohol and boiled for 10 minutes in water bath. The extracts were cooled in a pan of water and filtered through the two layers of cheese cloth. The residues were re extracted in the same way with 20-30 ml boiling 80% alcohol for 3 minutes, and filtered. This second extraction ensures complete removal of alcohol soluble sugar. The filtrates were pooled and centrifuged at 3000 rpm for 30 minutes, decant and supernatant was evaporated to one tenth of the total volume. The extraction was continued by addition of, a few ml of water till no reduction in the volume of the extract is noted. Extract were then adjusted to 10 ml by adding distilled water.

Three ml of aliquot of the extract were taken in test tubes having 3 ml of DNS reagent. The mixture then boiled for three minutes in a boiling water bath, and to this 1 ml of 40% solution of Rechelle salt (Sodium potessium tartarate) was added. The content in the tubes were then cooled under the running tap water and its absorbency was noted at 575 nm. Amount of reducing sugar was then calculated using standard curve prepared from D-glucose (AR grade) in the same manner.

2.4.5 Seed health test (germinability): Wheat grains were examined after 30 days and 60 days of storage, for their germinability following the paper top method as prescribed in the rules of ISTA (1982) and Shukla (1991). For this, 200 grains were shown on moistened filter paper pads in Petri plates. The plates were then incubated in seed germinator where 90-95% relative humidity was maintained

Observation for germination, were recorded after 7 days, the period after which no apparent change in percent seed germination was noted. The data for germination were classified under four categories i.e., (A) Seed germination normal without fungal infestation, (B) Seed germination normal, fungal infestation visible, (C) Abnormal germination (forming abnormal root and shoot or both and (D) Seed not germinating (Shukla, 1991).

2.4.6 Change in oil content of wheat during fungal infestation: Total fat is estimated as crude ether extract using the method of Raghuramulu et.al. 1983. For this, 10.0 g grains were soaked in diethyl ether, crushed and then allowed to stand with 100 ml of anhydrous diethyl ether at room temperature ($26^0C \pm 1^0C$) for 24 hours. The extracts were then filtered in to weighed conical flasks. The residue was thoroughly washed with 10-15 ml diethyl ether 3-4 times and the washing also transferred into the flasks. The ether is then evaporated and the flask with the residue were dried in an oven at 60^0C, cooled, desiccated and weighed. The percentage of ether extracted oil was calculated on the basis of dry weight of grain in each case.

2.4.7 Change in fibre content of wheat grain during fungal infestation: Fibre content of wheat grains was determined by the method as described by Raghuramulu et.al., 1983. For this, fat free 10 g oven dry grains were weighed in to a 500 ml beaker to this 200 ml of 0.255 N (1.25% w/v) H_2SO^4 (Sulphuric acid) was added. The mixture was boiled for 30 minutes, keeping the volume constant by addition of water at frequent intervals. After boiling, the mixture was diluted and filtered through muslin cloth. The residue was washed several times with hot water to make it acid free and transferred to a beaker having 200 ml of 0.313 N (1.25% w/v) NaOH. The content was boiled for 30 minutes (keeping volume constant as before). The mixture was diluted with hot water and filtered through muslin cloth to make residue alkali free. It was washed several time with hot water and then rewashed with some alcohol and ether. Finally, the content was dried at 80^0C, till constant weight and the values were calculated in terms of crude fibre (percentage) i.e. including ash content of grain samples using following formula:

$$\text{Crude fibre} = \frac{\text{wieght of the fibre}}{\text{weight of the sample}} \times 100$$

2.4.8 Assay of fungal lipases: (Test with chromogenic lipid substrates):
Production of lipases by test fungi was assayed using chromogenic lipid substrates such as p-nitrophenyl caprylate (C_8), p-nitrophenyl caprate (C_{10}), p-nitrophenyl laurate (C_{12}), p-nitrophenyl myristate (C_{14}), p-nitrophenyl pamitate (C_{16}) and p-nitrophenyl stearate (C_{18}) and by following the method of Yeoh et.al. (1986) and Shukla, (2014). For this, five ml of molten 4.5% (w/v) Czapek Dox Agar was allowed to solidified in the test tubes before an overlay of 0.5 ml of a mixture of 0.2 ml of 1% (w/v) lipid substrate dissolved in di-methyl sulphoxide (DMS) 2 ml of 100 mM phosphate buffer (pH 7.0) and 2 ml of molten 4.5 % (w/v) Czapek Dox Agar. The medium was allowed to solidify and then inoculated with 50 µl of spore suspension containing 2×10^6 spore /ml. The tubes were then incubated at $28^0C \pm 1^0C$ and $45^0C \pm 1^0C$ temperature. The presence of lipase activity as indicated by the appearance of yellow coloration in the tubes agar was recorded on an arbitrary scale after 3-5 days of incubation.

2.4.9 Degradation of outer pericarp of wheat by some Aspergilli : Fibre from wheat grains were separated by the method as described in the aforesaid experiments (G). The isolated fibre was sterilized by autoclaving and equilibrated. After autoclaving 10 g fiber were taken out from the bulk and dried in an oven at 80^0C till constant weight and the moisture content was calculated. Sterilized fibres were separated in four parts and water content was raised to 12%, 15%, 18% and 21% by adding appropriate amount of water to obtained required moisture content in fibre. After addition of water, the content of each flask was equilibrated at $2^0C \pm 1^0C$ temperature.10 gram fibres were taken in presterilized Erlenmeyer flasks of 150 ml capacity and inoculated with 1 ml spore suspension (having 2×10^6 spore/ml) of the test organism. The suspension was equilibrated by thorough shaking and then flasks inoculated with mesophilic test fungi were kept at $28^0 \pm 1^0C$ temperature. After 45 days the flasks were taken out and loss in weight of the fibre was calculated as described by Furgus (1969) for cellulolytic fungi.

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3. Results

3.1 Fungal contaminants of freshly harvested grains of wheat var WH147

In the present investigation unsterilized, untreated wheat grains were used. Before setting up of the experiments, these grains were tested by direct plating on two media for grain micro-flora and by blotter test to observe the percentage of germinating grains in them. The results of the study are given in Table 1. Maximum number of fungal contaminants were recorded from germinating grains when shown on blotters, these includes a total of 14 and 9 different fungal species from wheat grains. Wheat grains were found to contain hundred percent germinating grains including 28% grains which showed fungal infestation on them. Remaining germinating seeds were found without any fungal infestation on them (table-1). When these seed were plated in malt extract and malt salt agar media, a total of 8 and 10 fungi were recorded at 28^0C ± 1^0C. Absidia corymbifera, A. fumigates, A. niger A. terreus and Rhizomucor pusillus were the only thermophilous fungi which were recorded from wheat grain. However, these species were also recorded on the seeds when sown on blotters alongwith some other mesophilic fungi at28^0C±1^0C. It is point worthy to note that only 2% grains have showed no germination due to fungal infestation, although fungal was noted with 28% seeds.

Table 1: Fungal contaminants on freshly harvested grain of wheat variety WH 147

S.No.	*Name of the organism*	*Blotter test*	*Direct plating of grains on agar medium*			
			At 28^0C±1^0C temp.		At 45^0C±1^0C temp.	
			MS Medium	ME Medium	MS Medium	ME Medium
1	Absidia corymbifera	++	-	+	+	+
2	Alternaria altenata	++	+	+	-	-
3	Alternaria sp.	++	+	-	-	-
4	Aspergillus flavus	+++	+++	+	-	-
5	Aspergillus fumigatus	++	+++	+	+++	+++
6	Aspergillus niger	+++	-	+++	+++	+++
7	Aspergillus terreus	++	-	++	+++	++
8	Bipolaris australiensis	++	+	+	-	-
9	Cladosporium sp.	++	-	-	-	-
10	Curvularia sp.	++	+	-	-	-
11	Fusarium sp.	++	+	-	-	-
12	Helminthosporium sp.	+	+	-	-	-
13	Rhizomucor pusillus	+	+	+	+	+
14	Rhizopus sp.	+	+	-	-	-
Total		14	10	08	05	05
Percentage of infested seeds 28.0 %						
Percent of germinating seeds 100 %						

3.2 Change in amino acid in grains inoculated with test fungi: Wheat grains inoculated with A. flavus (M61) showed marked change in their amino acid contents in comparison to their initial concentration. Depletion of initial amount of L-arginine monohydrochloride, L-leucine and threonine were noted in A. flavus inoculated grains having 12% water content after 60 days incubation at 28^0C±1^0C temperature. A little decrease in the concentration of L-glutamic acid was also recorded in A. flavus inoculated grains (12.0% WC) but in all other grain samples its high concentration was noted at the end of 60 days. Amount of L histidine monohydrochloride also decreased in all A. flavus inoculated wheat grain samples.

DL-nor leucine showed modrate concentration in grain with 18% and 21% water content after 60 days while its high amount was recorded in grains of 9.99%,12% and 15% water content. DL-methionine was recorded in grains having 9.99% water content, while in all wetted grains, its presence was not observed, after 60 days of incubation. Grains with higher water contents were devoid of DL-metheonine even after 15 days of incubation. L- proline concentration varied in grains having different water contents.

A decrease in the initial amount of DL-tryptophane was also noted in all wetted grains after 60 days.

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Table 2: Qualitative change in amino acid content of wheat variety WH 147 following fungal infestation by two mesophilic fungi i.e. A. flavus (M61) and A. nidulans (M38) during 60 days storage at $28^0 \pm 1^0$C temperature

Amino acid	Concentration of amino acid# after 60 days of incubation												
	9.99%WC	12.0 % WC			15.0 % WC			18.0 % WC			21.0 % WC		
	Gran as such	Cont	M61	M38	Cont.	M61	M38	Cont.	M61	M38	Cont.	M61	M38
DL-2-Amino-n-butyric acid	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	1(In)	0(NC)	0(NC)	2(In)	2(Pi)
L-Arginine monohydrochloride	2(NC)	2(NC)	0(De)	2(NC)	2(NC)	2(In)	0(De)	2(NC)	2(In)	0(De)	0(NC)	3(In)	0(De)
DL-Aspartic acid	3(NC)	3(NC)	3(In)	2(NC)	3(NC)	2(Pi)	3(In)	2(Pi)	2(NC)	3(NC)	0(De)	2(Pi)	3(NC)
L-Cystein	3(NC)	3(NC)	3(In)	0(De)	3(NC)	3(In)	0(De)	1(De)	3(In)	0(NC)	0(De)	3(NC)	0(NC)
DL-3,4-dihydroxyphenyl alanine	0(NC)	0(NC)	0(NC)	1(NC)	0(NC)	0(NC)	1(De)	0(Pi)	0(NC)	0(NC)	0(Pi)	0(NC)	0(NC)
L-Glutamic acid	3(NC)	3(NC)	2(De)	2(De)	3(NC)	3(In)	1(De)	2(In)	3(In)	1(NC)	2(NC)	3(In)	1(NC)
L-Histidine monohydrochloride	3(NC)	3(NC)	2(NC)	3(NC)	3(NC)	1(De)	2(De)	0(De)	1(De)	1(De)	0(NC)	1(De)	1(De)
L-Leucine	3(NC)	3(NC)	0(De)	0(De)	3(NC)	2(In)	0(De)	0(NC)	2(In)	0(NC)	0(NC)	2(Pi)	0(NC)
DL-Nor leucine	3(NC)	3(NC)	3(NC)	0(NC)	3(NC)	3(Pi)	0(NC)	0(De)	2(De)	0(NC)	0(De)	0(De)	0(NC)
DL-Methionine	1(NC)	1(NC)	0(De)	1(De)	1(NC)	0(De)	1(De)	1(De)	0(NC)	2(In)	1(Pi)	0(NC)	1(De)
L-Proline	2(NC)	2(NC)	3(In)	2(NC)	2(NC)	2(De)	1(De)	3(In)	2(De)	1(De)	3(In)	3(In)	3(In)
DL-Serine	0(NC)	0(NC)	0(NC)	1(In)	0(NC)	0(Pi)	1(In)	0(NC)	0(De)	0(NC)	0(NC)	0(De)	0(NC)
DL-Threonine	1(NC)	1(NC)	0(NC)	2(In)	1(NC)	0(Pi)	2(In)	1(NC)	2(In)	2(NC)	1(NC)	2(In)	3(NC)
DL-Tryptophane	3(NC)	3(NC)	2(NC)	2(De)	3(NC)	2(De)	2(De)	3(NC)	2(NC)	2(NC)	3(NC)	1(De)	1(De)
L-Tyrosine	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	1(In)	0(NC)	0(De)	0(Pi)	0(NC)	0(NC)	1(In)
DL-Valine	1(NC)	1(NC)	0(NC)	0(De)	1(NC)	0(De)	0(Des)	1(NC)	0(De)	0(NC)	0(NC)	0(De)	0(NC)

Note: # Figures given in parenthesis indicate the pattern of concentration of amino acids in grain analyzed after 15, 30 and 60 days of incubation: NC- No change in concentration, De-Concentration decreasing, In-Concentration increasing and Pi-Pattern of change in concentration is irregular

Concentration of amino acids recorded on arbitrary scale by visual observation of intensity of colored spots on chromatogram: 1-poor, 2-moderate, 3-high and 0-not present.

Table 3: Qualitative change in amino acid content of wheat variety WH 147, following fungal infestation by two thermophilic fungi i.e. A. nidulans (TH24) and A. niger (TH147) during 60 days storage at 45^0C$\pm 1^0$C temperature

Amino acid	Concentration of amino acid# after 60 days of incubation												
	9.99%WC	12.0 % WC			15.0 % WC			18.0 % WC			21.0 % WC		
	Grain as such	Cont	TH 24	TH147	Cont.	TH 24	TH147	Cont.	TH 24	TH147	Cont.	TH 24	TH147
DL-2-Amino-n-butyric acid	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	0(Pi)	0(NC)	0(NC)	0(Pi)	1(De)	0(NC)	0(Pi)	0(Pi)
L-Arginine monohydrochloride	2 (NC)	2(NC)	1(De)	1(NC)	2(NC)	0(De)	0(De)	2(De)	0(De)	0(De)	3(Pi)	0(De)	0(NC)
DL-Aspartic acid	3(NC)	3(NC)	3(NC)	2(De)	3(NC)	2(De)	3(NC)	3(Pi)	2(De)	2(Pi)	3(Pi)	2(Pi)	0(De)
L-Cystein	3(NC)	3(NC)	1(De)	3(Pi)	3(NC)	1(De)	2(De)	3(NC)	1(De)	1(De)	3(NC)	2(De)	1(De)
DL-3,4-dihydroxyphenyl alanine	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	1(In)	0(NC)	0(NC)	1(In)	0(NC)	0(NC)	2(In)	0(NC)
L-Glutamic acid	3(NC)	3(NC)	2(De)	3(NC)	3(NC)	2(De)	3(NC)	3(NC)	2(De)	2(In)	3(NC)	2(De)	2(NC)
L-Histidine monohydrochloride	3(NC)	3(NC)	2(De)	2(De)	3(NC)	1(De)	0(De)	3(NC)	1(De)	0(De)	3(NC)	1(De)	0(De)
L-Leucine	3(NC)	3(NC)	2(De)	0(De)	3(NC)	2(De)	0(De)	3(NC)	3(Pi)	0(NC)	3(NC)	2(De)	0(NC)
DL-Nor leucine	3(NC)	3(NC)	3(NC)	3(NC)	3(NC)	3(NC)	2(De)	3(NC)	3(NC)	0(De)	3(NC)	3(Pi)	0(NC)
DL-Methionine	1(NC)	1(NC)	1(NC)	1(NC)	1(NC)	0(De)	0(De)	1(NC)	0(De)	1(In)	1(NC)	0(De)	1(Pi)
L-Proline	2(NC)	2(NC)	3(NC)	1(De)	2(NC)	2(De)	1(De)	2(NC)	2(De)	3(In)	2(NC)	2(Pi)	3(NC)
DL-Serine	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	0(Pi)	0(NC)	1(In)	0(De)	0(Pi)	1(In)	0(Pi)	2(In)
DL-Threonine	1(NC)	1(NC)	0(De)	1(NC)	2(In)	0(Pi)	1(Pi)	2(Pi)	0(Pi)	1(Pi)	2(In)	0(De)	1(Pi)
DL-Tryptophane	3(NC)	3(NC)	2(De)	3(NC)	3(NC)	1(De)	2(De)	3(NC)	1(De)	1(De)	3(NC)	1(De)	0(De)
L-Tyrosine	0(NC)	0(NC)	0(NC)	0(NC)	0(NC)	0(Pi)	0(De)	0(NC)	0(De)	0(De)	0(NC)	0(De)	0(NC)
DL-Valine	1(NC)	1(NC)	1(NC)	1(NC)	1(NC)	0(De)	0(De)	1(NC)	0(De)	0(De)	1(NC)	0(De)	0(De)

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Note: # Figures given in parenthesis indicate the pattern of concentration of amino acids in grain analyzed after 15, 30 and 60 days of incubation: NC- No change in concentration, De-Concentration decreasing, In-Concentration increasing and Pi-Pattern of change in concentration is irregular.

Concentration of amino acids recorded on arbitrary scale by visual observation of intensity of colored spots on chromatogram: 1-poor, 2-moderate, 3-high and 0-not present.

Change in amino acid content of molding wheat grain:
Change in uninoculated grains (grain control): Wheat grains having initial water content 9.99% showed high amount of DL-aspartic acid, L cystein, L-glutamic acid, L-histidine monohydrochloride, L-leucine, DL-nor leucine and DL-tryptophane.In addition to these, L-arginine monohydrochloride and L- proline were present in their moderate amount, and DL-metheonine, DL- threonine and DL- valine were found in poor amounts in the grain samples. No change in the concentrations of these amino acids was noted in grain when examined after 15, 30 and 60 days of incubation at $28^0C\pm1^0C$ temperature. On the other hand, grain having 12, 15, 18 and 21% WC indicated a change in the initial concentration during the long storage upto 60 days at both the test temperature. Presence of L- arginine in grain containing 21% water content could not be recorded after 60 days at $28^0C\pm1^0C$. While, the amount of L-cystein was also decreased and finally its poor concentration was recorded in 18% and 21% water content after 60 days.

Table 4: Change in amount of reducing sugars content of wheat variety WH 147, following fungal infestation.

Water content	Storage period	Amount of reducing sugars (mg)/g grain					
		Storage temperature $45^0C\pm1^0C$			Storage temperature $28^0C\pm1^0C$		
		Control	A.nidulans	A.niger	Control	A,nidulans	A.niger
Grain as such							
9.99% WC	30 days	1.64	NC	NC	1.78	NC	NC
	60 days	3.41	NC	NC	2.83	NC	NC
Wetted grains							
12.0%WC	30days	2.24	2.88	2.43	1.68	2.45	2.23
	60 days	3.29	4.19	5.24	1.99	3.67	4.19
15.0% WC	30 days	1.74	0.76	1.95	1.82	1.93	1.62
	60 days	3.35	8.92	3.56	1.67	4.40	5.03
18.0%WC	30 days	1.85	0.79	1.65	2.07	1.39	1.68
	60 days	2.41	3.98	5.14	3.45	5.45	4.19
21.0% WC	30 days	3.70	1.30	1.64	2.67	1.83	1.81
	60 days	5.45	3.68	4.40	1.88	4.82	3.88

Change in amino acids in grains inoculated with test fungi:
Wheat grains inoculated with A. flavus (M 61) showed marked change in their amino acid concentration in comparison to their initial concentration Depletion of initial amount of L- arginine monohydrochloride, leucine and DL-threonine, was noted in A. flavus inoculated grain with having 12% water content after 60 days of incubation at $28^0C\pm1^0C$. A little decrease in the concentration of L-glutamic acid was also recorded in A. flavus on 12% water content. But all other grain samples showed its high concentration after 60 days of incubation.

Change in reducing sugars of molding grain of wheat var.WH 147:
Quantitative changes in reducing sugar of wheat grain due to fungal infestation were tested by spectrophotometric method. The results of the analysis of reducing sugars in test wheat grain are given in table 4, in the beginning of the experiment the grains having 9.99% water content indicated presence of 1.41 mg reducing sugars per gram grain. A gradual increase in the amount of total reducing sugar in grain having 9.99% water content was noted after 30 and 60 days of their incubation at $45^0C\pm1^0C$ temperature.

Uninoculated wetted grain having 12%, 15%, 18% and 21% water content when examined after 30 and 60 days of incubation indicated a change in their total reducing sugar in comparison to grain as such (9.99% WC). Including some exceptions almost all inoculated and uninoculated (control) grain sample showed increased amount of reducing sugar after 60 days incubation then grain examined after 30 days of their incubation at both temperatures. Uninoculated grain having 15.0% and 21.0% water content (control) were found to contain less amount of reducing sugars after 60 days in comparison to grain examined after 30 days of incubation at $28^0C\pm1^0C$.

Aspergillus nidulans (TH 24) inoculated grains having 15% water content have indicated maximum amount of reducing sugars in grains after their 60 days storage. In addition to this, when these grain were tested after 30 days of incubation, showed the presence of minimum amount of reducing sugars i.e.,0.76 mg/g grain. Aspergillus niger (TH 147) inoculated grains having 12.0% and 18.0% water contents, have also indicated more amount of reducing sugars (i.e., 5.24 and 5.14 mg/g grain) then grain with having 15.0% and 21.0% water content, when tested after 60 days. Aspergillus flavus (M 61) and Aspergillus nidulans (M38) inoculated grains having 18.0% and 15.0%

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water content, respectively, indicated greater amount of reducing sugars in comparison to other test grain samples.

Table 5: Effect of incubation on seed germination of wheat var. WH147 following, fungal infestation after 30 days.

Water content of grains	Categories of germinating seeds	Percentage of seed/grain germination									
		Storage temperature 45^{0}C±1^{0}C					Storage temperature 28^{0}C±1^{0}C				
		Control	A.nidulans X	Y	A.niger X	Y	Control	A.nidulans X	Y	A.flavus X	Y
Grain as such											
9.99% WC	(A)	51					48				
	(B)	44					47				
	(C)	04					03				
	(D)	01					02				
Wetted grains											
12.0%WC	(A)	60	48	--	24	--	36	12	--	34	--
	(B)	38	06	02	21	--	54	58	04	46	02
	(C)	--	--	--	12	--	04	18	--	06	--
	(D)	02	08	--	22	--	06	08	--	12	--
15.0% WC	(A)	46	62	--	--	--	40	18	--	38	--
	(B)	44	15	01	24	--	48	58	--	54	--
	(C)	04	04	--	14	--	10	08	--	02	--
	(D)	06	18	--	60	02	02	16	--	--	--
18.0% WC	(A)	56	14	--	--	--	46	26	--	28	--
	(B)	44	60	--	--	--	48	48	02	48	02
	(C)	02	04	--	--	--	04	02	--	06	--
	(D)	02	22	--	90	10	02	--	02	16	22
21.0%WC	(A)	48	--	--	--	--	52	--	--	48	--
	(B)	50	--	--	--	--	40	30	--	08	02
	(C)	--	--	--	--	--	04	04	--	08	--
	(D)	02	100	--	94	02	04	66	--	36	--

Note: *Data indicates percentage of seed, **Categories of seed germination: (A) Normal seed germination without infestation, (B) Seed germination normal fungal infestation visible, (C) Forming root/shoot (Abnormal germination), (D)Seed not germinating.
X- Inoculants and Y- other fungi.

Table 6: Effect of incubation on seed germination of wheat var.WH147 following fungal infestation after 60 days

Water content of grains	Categories of germinating seeds	Percentage of seed/grain germination									
		Storage temperature 45^{0}C±1^{0}C					Storage temperature 28^{0}C±1^{0}C				
		Control	A.nidulans X	Y	A.niger X	Y	Control	A.nidulans X	Y	A.flavus X	Y
Grain as such											
9.99% C	W(A)	70					80				
	(B)	28					18				
	(C)	02					02				
	(D)	--					02				
Wetted grains											
12.0% WC	(A)	78	08	--	10	--	56	08	--	10	--
	(B)	18	70	10	56	14	44	82	28	64	20
	(C)	04	10	--	16	04	--	08	--	22	--
	(D)	--	02	--	--	--	--	02	--	02	--
15.0% WC	(A)	70	04	--	06	--	50	06	--	02	--
	(B)	26	66	04	12	02	48	70	02	50	--
	(C)	02	18	--	58	--	02	22	--	46	--
	(D)	02	08	--	22	--	--	02	--	02	--
18.0% WC	(A)	54	08	--	10	--	60	08	--	30	--
	(B)	44	60	02	36	--	38	54	02	22	02
	(C)	02	24	--	34	--	--	24	--	36	--
	(D)	--	06	--	20	--	02	14	--	12	--
21.0% WC	(A)	54	14	--	10	--	62	12	--	42	--
	(B)	46	46	--	54	--	34	44	--	22	--
	(C)	--	30	--	22	--	02	36	--	28	--
	(D)	--	10	--	14	--	02	08	--	08	--

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*Data indicates percentage of seed,
**Categories of seed germination:
(A) Normal seed germination without infestation,
(B)Seed germination normal fungal infestation visible,
(C)Forming root/shoot (Abnormal germination),
(D)Seed not germinating.
X- Inoculants and Y- other fungi.

4. Discussion

Deterioration of wheat grains due to fungal infestation was investigated in present study. All the test fungi when allowed to grow on wheat grains having different water content, have altered the reducing sugar content per gram grains when compared with control i.e., unwetted, uninoculated grains. Except few cases, inoculated wheat grains having different water contents showed increased amounts of reducing sugar per gram test grain samples then their respective grain controls having similar water content. An increase amount of total reducing sugars in inoculated wetted grains may be due to the activity of fungal contaminants which were initially present in them (Table 4**)** while increase in sugar content in inoculated grains is a result of the activity of inoculants and other initially present contaminants of these grains.

Fungal organisms are highly specific for their nitrogen requirements (Bilgrami & Verma, 1978). Most investigations are more or less agree that glycine, aspartic acid, asparagines and glutamic acid are the best source of nitrogen for many fungi but exceptions to these findings are also known (Cochrane,1958). However, when a fungus grows on a natural substrate, it produces proteolytic enzyme to convert the complex proteins into simple amino acids to fulfill its nitrogen requirement. The liberated amino acids in the surrounding medium are readily utilized by the growing fungus but depletion of most preferred amino acids will be more rapid from the hydrolysate then others during early phase of fungal growth. Presence of butyric acid, phenylalanine, serine and tyrosine were not recorded in the wheat grains in the beginning of the experiments, but their appearance was noted in some of the inoculated and uninoculated wetted grains during 60 days of incubation at $28^0C\pm1^0C$ and $45^0C\pm1^0C$. Initially present arginine, histidine, metheonine, threonine and valine could not be detected in A. nidulans and A.niger inoculated grains having different water contents similar observations with other amino acids have also been recorded when wheat grains were inoculated with mesophilic test fungal strains.

Change in free amino acids in inoculated and uninoculated wetted grains having different water contents indicated a result of complex microbial activity on wheat grains. The presence of amino acids in grain extract after a particular time depends on two processes (i) Liberation of amino acids from grain proteins by the activity of proteolytic enzymes produced by the infesting fungus and (ii) Their extracellular production of amino acids by the growing fungal mycelium on grains. While absence of amino acids from grain extracts indicates their utilization by infesting fungi for their growth, development and for the synthesis of secondary metabolites. Adey and Mateles (1964) demonstrated the incorporation of tryptophane into aflatoxin B_1, and concluded tryptophane to act as precursor in aflatoxin synthesis, but Mateles and Wogan (1967) have dis-approved the above suggestion. Several workers used labeled metheonine and observed that metheonine specifically labeled the methoxy group of aflatoxin (Adye and Mateles, 1964 Holker and Underwood, 1964; Biollaz et. al. 1968; Shukla, 2014). Partial incorporation of leucine and isoleucine into aflatoxin has also been reported by Heathcote et. al.(1973). Adye and Mateles (1964) have also demonstrated the incorporation of ^{14}C labeled phenylalanine and tyrosine into aflatoxin B_1 and suggested that since aflatoxin is a cumarin derivative, the cumarin skeleton of aflatoxin is formed from phenylalanine and tyrosine, which are shikmic derivatives. Depletion of some of amino acids from wheat grains is also indicating the late requirements of these amino acids by growing fungi for synthesis of some secondary metabolites.

In addition to amino acids, carbohydrates are also reported important nutrients in relation to biosynthesis of secondary metabolites by fungi (Diener and Davis, 1969). Cereal grains which used by fungi as substrates, are rich in starch similarly influence the production of mycotoxins and other secondary metabolites by contaminating fungi (Wyllie and Morehouse, 1977; Prasad, 1983; Shukla 1991).

Fungal infestations have also caused a loss of grain germinability. Inoculated wheat grains have lost their germinability to a greater extent, when moisture content of grain was raised to 21%. However, the grains having low moisture content i.e. 12% and 15% have showed greater infestation by contaminating fungi, the net loss in germinability was found to be less in wheat grains when observed after 60 days. One of several causes of decrease in germination percentage of seeds during storage is invasion of the embryos by storage fungi (Neergaard, 1977). In present study all the four test fungi have found to decrease the germination percentage of wheat grains. Aspergillus flavus and A.niger have been found as a germ colonizer of wheat grain, while strains of A. nidulans were found to attack whole kernel in the initial stage of colonization. A. nidulans also attack germ portion of wheat kernel, while studying the effect of various factors upon mould invasion and germination of stored wheat. Wyllie and Christensen (1959) reported increased deterioration of wheat grains with the increase in moisture content, temperature and time duration. Results of the present study also indicate the similar influence of moisture content, time of incubation and temperature on germination of inoculated wheat grains. Test thermophilous fungi caused more damage in wheat grains almost of all the different water contents tested (12%, 15%,18% and 21%) than mesophilic test strains, when they were allowed to grow at $45^0C\pm1^0C$ and $28^0C\pm1^0C$ temperature, respectively.

In the present study the test fungi were also assayed for the production of lipases responsible for hydrolysis of fatty acid esters of different carbon chain length. All the test fungi have indicated the production of lipases responsible for hydrolysis of C_8,C_{10} and C_{12} carbon chain length fatty acid esters. However, test strains showed poor to moderate

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activity of lipases C_{14},C_{16} and C_{18} carbon length fatty acids. Production of different lipases by the test fungi supports their possible role in the wheat germ damage during storage. Percentage oil yield was also found change in inoculated wheat grains in comparison to uninoculated grains as such, indicating the activity of inoculated fungi on lipid constituent of wheat grain (Shukla, 2014).

Degradation of wheat bran by test strains of the genus Aspergillus indicated their possible role in deterioration of wheat grain and a loss of bran quality due to these fungi during their storage. Bran has its own importance in food preparations, particularly in bakery preparations. Whole bran cakes are sold with high prices and as a corrective dietary supplement in all over the world market.

5. Conclusions

A perusal of the data of various studies conducted in the present research work confirms the damaging effect of all the test fungi on wheat grains of different water contents during storage. In addition to this, these fungi indicated their possible multidirectional deteriorative effect on grain quality and quantity. However, experimental grains having more water content were found most susceptible for incidence of test fungi than those having less water content and hence proper drying of grains to the level of minimum water content prior to their storage is suggestive. This approach will ensure the safe storage and minimize the loss of grains during storage and also minimize the loss of seed germinability, and can maintain their quality even under prolonged period of storage.

References

[1] Adey, J..and Mateles, R.I. (1964). In corporation of labeled compound into aflatoxins. Biochem. Biophysics Acta. 86: 418- 420.
[2] Bilgrami, K.S. and Verma, R.N. (1978). In: Physiology of fungi. Vikas Publishing House, New Delhi. Pp 240.
[3] Biollaz, M., Buchi,G. and Milne,G. (1968).The biogenesis of the bisfuranoids in genus Aspergillus. Journal of American chemical soc., 90: 5019-5020.
[4] Chritensen,C.M.and Kaufman, H.H. (1968) Maintenance of quality in stored grain seeds. Agricultural extension service. University of Monnesota, Extension folder, 226:1-4.
[5] Chritensen, C.M. and Kaufman, H.H. (1969) Grain storage: The role of fungi in quality loss. University of Monnesota Press, Minncapolis.
[6] Christensen, C.M. (1971) Invasion of sorghum seed by storage fungi at moisture content 13.5-15.0% and condition of samples from commercial bins. Mycopth. Myco. Appl., 44: 277-282.
[7] Christensen, C.M.(1978) Storage fungi. In : food and beverage. Mycology (ed.) L.R. Beuchat, 7: 173-190.
[8] Christensen,C.M. and Gordon, D.R. (1948) The mould flora of stored wheat and corn and its relation to heating of moist grain. Cereal Chem., 25:42-51.
[9] Christensen,C.M. and Lopez, F.L.C. (1963) Pathology of stored seeds. Proc. Int. seed test Asso., 27: 701-711.
[10] Cochrane, V.W. (1958). Physiology of fungi. J.Willey, New York.pp 75-79.
[11] Corry, J.E.L. (1978) Relationship of water activity to fungal growth. In: Food and beverage mycology. (ed.) L.R.Beuchat. AVI Pub. Co. Inc ., West Port, Conneticut. Pp 45-82.
[12] Diener, U.D. and Davis, N.D. (1969) In aflatoxin. (ed.) L.A. Goldblatt. ACADEMIC Press, New York. Pp 25.
[13] Flannigan, B. (1974) Distribution of seed-borne microorganisms in naked barley and wheat before harvest. Trans. Brit. Mycol. Sos., 62: 51-58.
[14] Flannigan,B. (1978) Primary contamination of barley and wheat grain by storage fungi. Trans. Brit. Mycol. Sos., 71(1)37-42.
[15] Heathcote, j.g. and Hibberts, J.R. (1978) Aflatoxins : Chemical and biological aspects. Elsevier scientific publishing house co. Amsterdam, Oxford, New York. Pp 212.-214.
[16] Holker, J.S.E. and Underwood, J.G. (1964) Chem. Engl. 1865-1866.
[17] ISTA (1982) International rules for seed testing. Proceeding Int. seed Test. Assoc. 31:1-152.
[18] Lacey,J. (1975) Moulding of grain in relation to mycotoxins formation. International Journal of Environmental studies, 8:183-186.
[19] Lacey, J. (1989) Seed storage : An exercise in ecological management. Proc. Nat. Sem. Advan. In seed sci.and tech. pp 324- 335.
[20] Mahadevan,a. and Sridhar, R. (1982) Methods in physiological plant pathology, IIedition. Sivakami publication, Madras.
[21] Mills, J.T. and Wallace, H.A.W. (1979) Microflora and conditions of cereal seeds after a wet harvest. Canadian J of plant sci., 59:645-651.
[22] Neergaard, P. (1977) Seed pathology. Macmillan press Ltd. London, UK, pp 282- 297.
[23] Prasad, R.B. (1983) Influence of carbon source on growth and aflatoxins production by Aspergillus parasiticus (NRRL-3240) Proc. Symp. Mycotoxins in food and feed (ed) K.S. Bilgrami, 207-215.
[24] Raghuramulu, N.;Madhavan, K. Nair and Kalyanasundaram, S. (1983) Amanual of laboratory techniques. National Inst. Of Nutrition, ICMR, Hyderabad.
[25] Qasem, S.A. and Chritensen, C.M. (1958) Influence of moisture content, temperature and time on the deterioration of stored corn by fungi. Phytopathol. 48: 544-549.
[26] Tuite, J.F. (1959) Low incidence of storage molds in freshly harvested seed of soft red winter wheat. Plant Disease Reporter. 43: 470-474.
[27] Tuite, J.F. (1961). Fungi isolated from unsaturated corn seed in Indiana. In : 1956-1958. Plant disease research, 45: 212-215.
[28] Warnock, D.W. and Preece, T.F. (1971) Location and extent of fungal mycelium in grains of barley. Trans. Brit. Myco. Soc., 56:257-273.
[29] Willing, B.(1974) Aspergillus and Penicillium species on stored Danish barley grain. Identification of species of fungi isolated from 40 barley samples in the harvested year 1970. Tidssk pl. AVL., 78: 1-8.

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[30] Sinha, R.N. (1973) Ecology of storage. Annals de techonologie agricole, 22: 351-369.
[31] Shukla, A.K. (1991) Spoilage of wheat and sorghum produced by thermophilous fungi during storage. Ph D Thesis, Dr, H.S.Gour Vishwavidhyala Sagar, India.
[32] Shukla, A.K. (2014) Biodiversity in Aspergillus nidulans group on the basis of lipases profile. **http://www.ijsr.net, ISSN: 2319-7064, Volume-3, Issue-6, pp1391-1394**. (Impact factor- 3.358), **Paper ID-02014487**
[33] Shukla, A.K. (2014) Degradation of timber wood by filamentous fungi. **http://www.ijsr.net, ISSN**: **2319-7064, Volume-3, Issue-7, pp1091-1094**. (Impact factor- 3.358), **Paper ID-020141184**
[34] Shukla, A.K.(2014) Extracellular production of thermostable endoglucanase by thermophilous fungi. **http://www.ijsr.net, ISSN: 2319-7064, Volume-3, Issue-8, pp 110-113.** (Impact factor- 3.358), **Paper ID-020151165**
[35] Shukla, A.K. (2014) Occurrence of keratinophilic fungi from the soils of Chhattisgarh. **http://www.ijsr.net, ISSN: 2319-7064, Volume-3, Issue-9, pp 2041-2044.** (Impact factor- 3.358), **Paper ID- SEP14574**
[36] Shukla, A.K. (2014) Occurrence of mycotoxins in storage fungi. **http://www.ijsr.net, ISSN: 2319-7064, Volume-3, Issue-11, pp 1747-1750.** (Impact factor- 3.358), Paper ID-OCT 14188
[37] Shukla, A.K.& Singh, Annu (2014) Diversity of forest of Surguja District Chhattisgarh, India**. http://www.ijsr.net, ISSN: 2319-7064, Volume-3, Issue-6, pp1153-1157**. (Impact factor- 3.358), **Paper ID-SUB 14653**
[38] Stahl, E. (1965) Thin layer chromatography- a laboratory hand book, Academic press, New York.
[39] Wyllie, T.D. and Morehouse, L.G. (1977) Mycotoxic fungi, Mycotoxins, Mycotoxicoses. An encyclopedic hand book. Mycotoxic fungi and chemistry of mycotoxins. Vol. I (Eds.) T.D. Wyllie and L.G. Morehouse pp 538.
[40] Wyllie, T.D. and Christensen, C.M. (1959) Influence of moisture content and temperature upon mold invasion and germination of stored wheat. Plant Disease Reporter 43(7): 764- 767.
[41] Yoeh,H.H., Wong,F.M. and Lim, G. (1986) Screening of fungal lipases using chromogenic lipid substrates. Mycologia, 78(2): 298-300

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