ISOLATION AND IDENTIFICATION OF SOME PATHOGENIC FUNGI ASSOCIATED WITH STORED BARLEY GRAINS IN ZLITEN AREA, LIBYA

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ABSTRACT

The deterioration of barley grains during their storage by fungi has been a problem of economic importance, especially in the use of grains barley for soowinging and other uses. Results of the present study could be summarized in the following points, the isolated fungi were differed considerably in their number and kinds according to the inspected locality. Generally, the isolated fungi from stored barley grains were represent 10 species belonging to 6 genera. These fungi were purified and identified as Aspergillusflavus, Aspergillusochraceus, Aspergillussydowii, Chaetomiumglobosum, Cladosporiumcladosporioides, Eurotiumarnstelodami, Eurotiumintermedium, E. repens, Penicilliumchrysogenum, and Rhizopusstolonifer. The most frequent fungi were Aspergillusflavus and Rhizopusstolonifer. On the other hand, Cladosporiumcladosporioides, Eurotiumintermedium, Chaetomiumglobosum and Eurotiumarnstelodami were less frequency. The pathogenicity test using healthy barley grains and 5 different of isolated fungi showed that Aspergillusflavus, followed by Aspergillusochraceus and Rhizopusstolonifer were significantly more pathogenic compared with the control as they decrease grain germination percentage to 33.3% for each one and they caused 100.0 , 93.3 and 93.3% of kernel invasion, respectively. On the contrary, Aspergillussydowii and Penicilliumchrysogenum were the least pathogenic. The analogous values for grain invasion and germinability were 100.0 and 60.0% for each one. However, the difference between any treatment and the control was significant.

Keywords: About four key words or phrases in alphabetical order, separated by commas.

1. INTRODUCTION

Barley is liable to be attacked with many diseases caused by fungi, i.e. leaf rust, powdery mildew, net blotch, grain rot during storage and other diseases of minor importance (Mc Donald and Buchannon, 1964).

Seed-borne fungi are chiefly responsible for deterioration of seeds in stores (Lutey& Christensen, 1963; Christensen & Kaufmann, 1965) and thus, they remarkably reduce the germination potential of stored seeds.

Storage conditions are very important factors affecting grain quality and quantity (Christensen, 1982). Under suitable conditions for mold growth, all barley grains will be damaged by fungi (Lacey and Magan, 1991). Some molds are toxigenic under favorable conditions (Mills, 1990).

The use of fungicides is still for the control of plant pathogens, but is being discouraged due their toxicity to human health and the environment pollution. A distinct group of pest control agents is the microbial pesticides including bacteria, viruses and fungi. Their use is often called "biological control.

The rapid growth of Trichoderma gives it an important advantage in the competition for space and nutrients with plant pathogenic fungi (Simon, C. and Sivasithamparam, M. (1988).

2. LITERATURE REVIEW

Nowadays, post harvest diseases are responsible for some of the most severe crop production losses, especially, in the developing countries. More than of 150 species of fungi have been reported on cereal grains (Christensen and Kaufmann, 1969 and Ichinoe et al., 1973). Damage by fungi can substantially reduce quality, grade and price of cereal grains and their products (Meronuck, 1983).

The fungi associated with grains were divided into two groups: field fungi and storage fungi. Field fungi invade the developing or mature grains while it is still on the plant. The major genera are Alternaria, Cladosporium,

Drechslera, Epicoccum, Helminthospor-ium, Mucor and Stemphylium. Storage fungi usually invade the grains during storage. These fungi include Aspergilluscandidus, A. flavus, A. fumigates, A. glaucus group, A. niger and A. versicolor. Also, the same author reported that there is no one medium or technique sufficient to disclose all of the organisms that might be present in a given lot of grains. For storage fungi, malt salt agar, consisting of 1-2% malt extract, 2% agar and 7.5-20% sodium chloride, may be the best one to get a large number of these fungi. (Christensen, 1957).

3. METHODS

3.1 Seed sampling:

Samples of stored barley grains were collected in January, 2013 from four different localities at Zeletin City, i.e. Dafniya, Swaih, Soufijeen and Mymoon. All stored seeds samples were obtained according to the rules of International Seed Testing Association (ISTA, 1966) as follows; Several individual primary samples were drawn from different parts of bags. Each of these were gathered together to be combined samples.

3.2 Isolation and identification of associated fungi:

Fifty grains of each sample were randomly chosen to isolate the different accompanied fungi as follows:

The grains were disinfected in 2% sodium hypochlorite solution for 2 mins; then rinsed 2 time in sterilized distilled water and dried on sterilized filter paper.

Malt Salt Agar medium (MSA) according to Christensen and Meronuck (1986) was used for isolation trials. Rose Bengal stain (1% of 10% solution) was used to avoid bacterial growth. Five replicated dishes were

applied for each sample (10 grains / plate) under aseptic conditions. The plates were incubated at 25 ± 2°C for 10 days and the ernerged fungi were picked up, periodically, on PDA slants.

The identification of isolated fungi was as verified on 10-days old cultures at the Faculty of Science, Mycological Center-Assiut, Egypt. The percentage of frequency for all the identified fungi was also calculated.

3.3 Pathogenicity test:

The pathogenic capability of five different fungal isolates which were previously isolated in high number from stored barley grains was carried out in vitro, The tested fungal isolates were consist of one isolate of each of Aspergillusochraceus, Aspergillussydowii, Aspergillusflavus, Penicilliumchrysogenum and Rhizopusstolonifer.

3.4 Preparation of barley grains:

Barley grains, apparently disease and insect-free were disinfected by immersing into 2 % sodium hypochlorite solution for 2 mins., then washed 3 times in sterilized distilled water and dried. Grains were stored in sterile glass containers till use (Christensen, 1957).

3.5 Preparation of spore suspension:

The spores of each tested fungal isolate were liberated using sterilized needle by scrapping the surface of 10- days old cultures grown on Potato Dextrose Agar (PDA) medium. The spores were suspended into sterilized 0.1% agar solution to avoid spore agglutination. The inoculum density was determined by a haemocytometer slide and adjusted to contain 10^5 spores/ml.

3.6 Determination of grains moisture content:

Five grams of barley grains were ground in a blender and dried in an oven at 130°C for 20 hrs, then grains reweighed and the moisture content was calculated according to Stroshine et al., (1984) as follows:

$$\text{Moisture content \%} = \frac{b-a}{b} \times 100$$

Where:

a= Weight of the sample after drying.

b= Weight of the sample before drying.

For adjusting moisture content to any required level, the following formula proposed by Fahmy, (1960) was used.

4. RESULTS AND DISCUSSION

Fungal isolation from stored barley grains using MSA medium:-

The isolation trials from surface sterilized barley grains yielded 10 species of fungi belonging to 6 genera (Table l and, Fig.1). These fungi were purified and identified as Aspergilliusflavus, A. ochraceus, A. sydowii, Chaetomiumglobosum, CladosporiumCladosporioides, Eurotiumamnstelodami, Eurotiumintermedium, Eurotiumrepens, Penicilliumchrysogenum and Rhizopusstolonifer, The most frequent fungi were Aspergillusflavus and Rhizopusstolonifer. Their correspoding frequencies were 19.64% for each fungus Aspergillusochraceus and Eurotiumrepens were the second in this respect.

Their analogous frequencies were 12.5% for each one. On the other hand, each of CladosporiumcladosporioidesmEurotiumintermedium,

Chaetomiumglobosum and Eurotiumarnstelodami were less frequency, being 3.57, 3.57, 1.78 and 1.78%, respectively.

Also, data show that the total count of fungal isolates infecting stored barley grains was variable from one locality to another where the highest number of isolated fungi, i. e. 42 isolates was obtained from barley grains collected from Dafniya locality, while the lowest number (22 isolates) was found in the grains collected from Soufijeen locality. This might be due to the high moisture content of the grains and /or their storage for a given period. Other two localities, i.e. Mymoon and Swaih gave the intermediate count in this concer, being 23 and 25 isolates, respectively. In general, it could be concluded that the isolation trials led to obtain a high numbers of storage fungi (e.g. Aspergillus and Penicillium) if compared with that of the field fungi (e.g. Chaetomium and Eurotium). The Aspergillus isolates numbers were more than that recorded for Penicillium ones. This means that the majority of inspected samples were stored for different periods before the isolation process.

The obtained data are somewhat in agreement with those recorded by Tuite and Christensen (1955). and Qasem and Christensen (1958). They stated that in stored barley, spoilage is often attributed to the growth of storage fungi, especially strains of Aspergillus and Penicillium. Also Parde et al., (2004). showed that Penicillium spp. and Aspergillusversicolor comprised the predominant microflora on barley grains. On the contrary, the obtained data are not in agreement with those recoroded by Tsuruta, (1974) and McGimpsey and Malone (1981). They observed that the isolates fungi from stored barley grains were species of Aspergillus, Epicoccum, Fusarium, Cladosporium, Mucor, Rhizopus and Penicillium.

Al-Aswed, (2010) reported that barley grains contained 20 identified species related to 14 fungal genera, mainly Penicillium spp., fellowed by Aspergillusniger. There was a high significant difference in the isolated organisms and tested samples. The difference between these results could be due to cultivar tested, grains situation (sterilized or unsterilized), type of medium number of inspected samples and / or the storage period.

The predominance of any fungus might be due to the possibility of antagonism between this fungus and other ones.

This experiment was conducted to give some light on the role of the isolated fungi in barley grains deterioration during storage. The obtained data are presented in Table(2) and Fig.(2). It was noticed that the tested fungi were significantly differed in their ability to colonize the intact barley grains or their effect on grain germinability if compared with the control treatment (the uninoculated grains). A. flavus, followed by A. ochraceus and R. stolonifer proved to be highly pathogenic. The percentage of kernel invasion induced by these fungi was 100.0, 93.3 and 93.3%, respectively accompanied with 33.3% germination for each fungus. Meanwhile, A. sydwii and P. chrysogenum were the least effective on either invasion or germination percentage of barley grains, being 100.0 and 60.0, for each one.

5. CONCLUSION

From this study, ten pathogenic fungi were revealed to be associated with barley grain diseases namely: Aspergillusflavus, Aspergillusochraceus, Aspergillussydowii, Chaetomiumglobosum, Cladosporiumcladosporioides, Eurotiumarnstelodami, Eurotiumintermedium, E. repens, Penicilliumchrysogenum, and Rhizopusstolonifer.

TABLE (1): FUNGI ASSOCIATED WITH STORED BARLEY GRAINS, COLLECTED FROM 4 DIFFERENT LOCALITIES AT ZELETIN CITY*

ISOLATED FUNGI	NUMBERS AND FREQUENCY OF ISOLATED FUNGI FROM 4 DIFFERENT LOCALITIES				TOTAL NO. OF ISOLATES	FREQUENCY, %
	DAFNIYA	MYMOON	SOUFIJEEN	SWAIH		
ASPERGILLIUS FLAVUS	6	3	10	3	22	19.6
ASPERGILLUS OCHRACEAS	2	7	2	3	14	12.5
ASPERGILLUS SYDOWII	8	-	-	2	10	8.9
CHAETOMIUM GLOBOSUM	-	-	2	-	2	1.8
CLADOSPORIUM CLADOSPORIOIDES	1	-	-	3	4	3.6
DARK STERILE MYCELIUM	2	1	-	5	8	7.1
EUROTIUM ARNSTELODAMI	-	-	2	-	2	1.8
EUROTIUM INTERMEDIUM	-	3	-	1	4	3.6
EUROTIUM REPENS	3	3	3	5	14	12.5
PENICILLIUM CHRYSOGENUM	-	8	1	1	10	8.9
RHIZOPUS STOLONIFER	20	-	2	-	22	19.6
TOTAL NUMBER OF ISOLATES	42	25	22	23	112	-

*** TOTAL NUMBER OF THE TESTED GRAINS WAS 50 / EACH LOCALITY.**

TABLE (2): PATHOGENICITY TEST OF 5 DIFFERENT FUNGAL ISOLATES USING BARLEY

GRAINS AFTER 30 DAYS OF INCUBATION AT 25 ± 2°C.

TESTED FUNGAL ISOLATES	AVERAGE PERCENTAGE OF GRAIN INVASION *	AVERAGE PERCENTAGE OF GRAIN GERMINATION
ASPERGILLUS SYDOWII	100.0	60.0
ASPERGILLUS FLAVUS	100.0	33.3
ASPERGILLUS OCHRACEUS	93.3	33.3
PENICILLIUM CHRYSOGENUM	100.0	60.0
RHIZOPUS STOLONIFER	93.3	33.3
CONTROL(UNIMOCULATED GRAINS)	4.0	94.0
L. S. D. AT 5%	13.3	49.7

* Each figure represents of 5 replicates.

Figure and legends

Fig.(1) Frequency percentage of fungi associated with stored barley grains using MSA medium, 10 days after incubation at 25 ±2° C.

Fig. (2): Pathogenicity test of some isolated fungi from stored barley grains after 30 days of incubation at 25 ± 2°C.

This experiment was conducted to give some light on the role of the isolated fungi in barley grains deterioration during storage. The obtained data are presented in Fig.(2). It was noticed that the tested fungi were significantly differed in their ability to colonize the intact barley grains or their effect on grain germinability if compared with the control treatment (the uninoculated grains). A. flavus, followed by A. ochraceus and R. stolonifer proved to be highly pathogenic. The percentage of kernel invasion induced by these fungi was 100.0, 93.3 and 93.3%, respectively accompanied with 33.3% germination for each fungus. Meanwhile, A. sydwii and P. chrysogenum were the least effective on either invasion or germination percentage of barley grains, being 100.0 and 60.0, for each one

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FIGURE 1

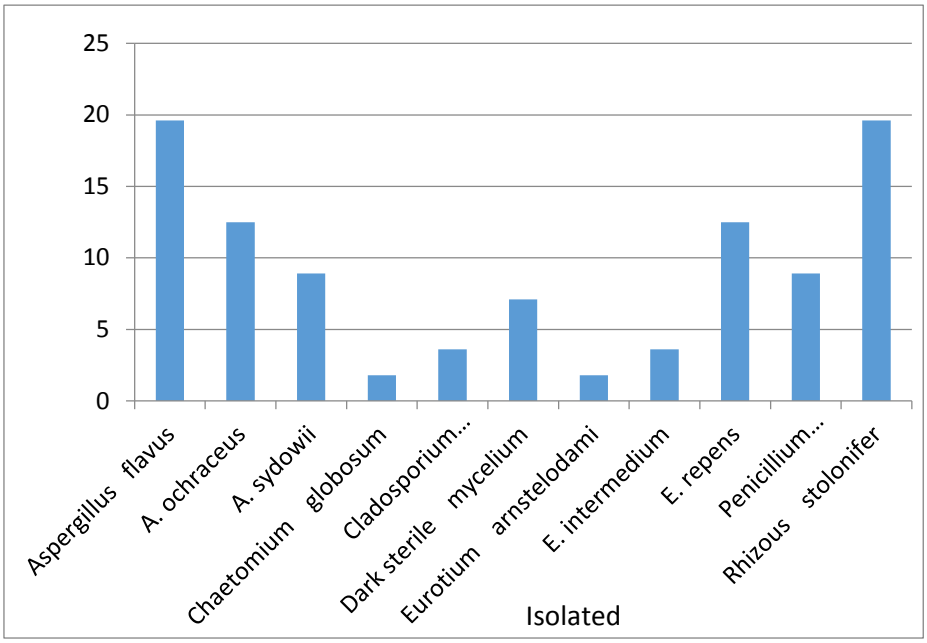

Fig.(1) Frequency percentage of fungi associated with stored barley grains using MSA medium, 10 days after incubation at 25 ±2° C.

FIGURE 2

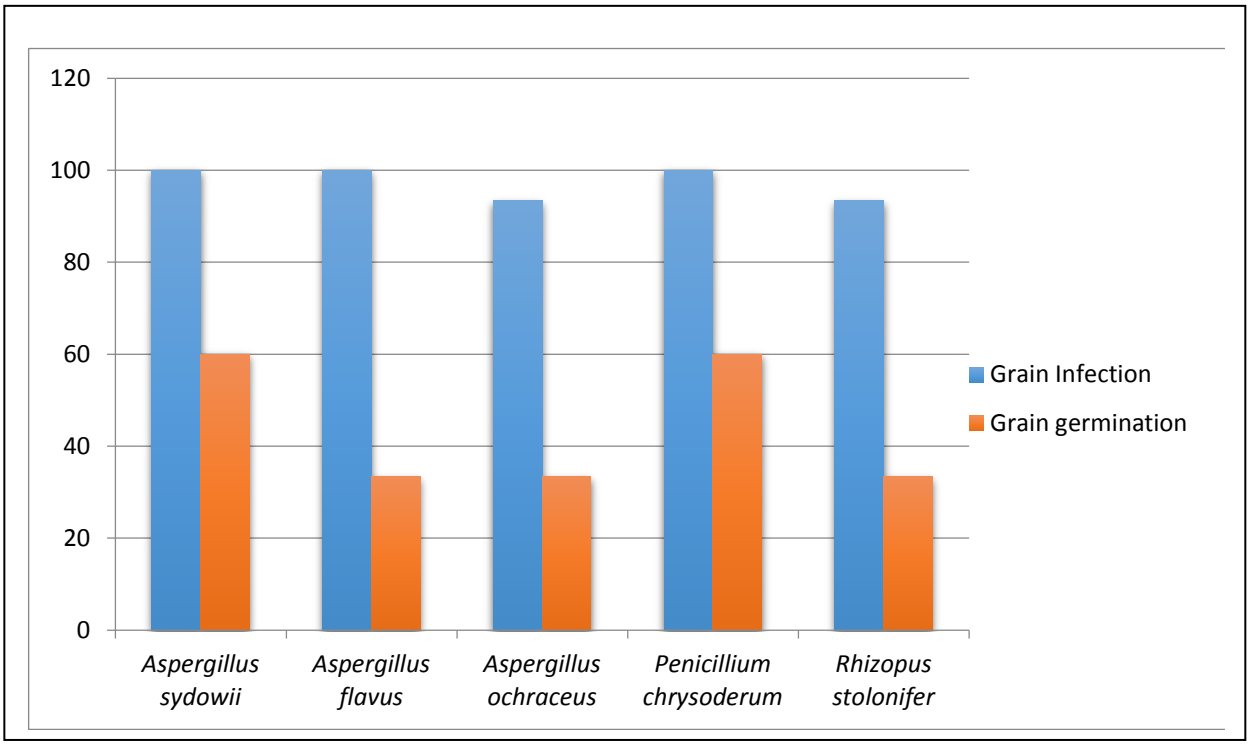

Fig. 2. Magnetization as a function of applied field. Note that "Fig." is abbreviated. There is a period after the figure number, followed by two spaces. It is good practice to explain the significance of the figure in the caption.

REFERENCES

[1] Abdel-Hafez, S.I.I., (1984). Composition of the fungal flora of four cereal grains in Saudi Arabia. Mycopath., 85 (12): 53-57.

[2] Ahmed, S..M.F., (1971). Deteriortion of corn grains in storage by fungi. PhD. Thesis. Fac. Of Agric., Ain Shams Univ., Cairo.

[3] Al-Aswed,. N. M. (2010). Isolation and identification of ochratoxin A producing fungi in corn and barley and antagonison with atoxigenic fungal isolates. M . Sc. Thesis. Faculty of Arts and Science. Al-Mergeb University.

[4] Armolik, N.;G.J. Dickson and A.D. Diskson (1956). Deterioration of barley in storage by microorganisms. Phytopath., 46: 457-461.

[5] Briggs, E. D. (1978). Barley. 8. The reception and storage of whole plants and grain. Chapman & Hall. A Halsted Press Book, John Wiley &Sone, New York, 417 pp.

[6] Calistru, C., Mclean, M. and Berjak, P. (1995). Some aspects of the biological control of seed storage fungi. In Basic and Applied Aspects of Seed Biology. Proceeding of the Fifth International Worvshop on Seeds in South Africa.

[7] Chet, I., Henis, T. and Mitchell, R. (1967). Chemical composition of hyphal and sclcrotial walls of SclerotiumrolfsiiSacc Can. J. Microbiol., B: 137-141.

[8] Christensen, M.C. (1957). Deterioration of stored grains by fungi. Botanical Review, 23: 108-134.

[9] Christensen, M. C. (1982). Storage of cereal grains and their products, American Association of Cereal Chemists Inc., St. Paul, MN 683 pp.

[10] Christensen, M. C. and Dreschler, R. F. (1954). Grain storage studies. XIV. Changes in moisture content, germination percentage, and moldiness of wheat samples stored in different portions of bulk wheat in commercial bins. Cereal Chem., 31: 206-216.

[11] Christensen, M. C. and Kaufmann, H. H. (1965). Deterioration of Stored grain by fungi. Ann. Rev. Phytopath., 3, 69-84.

[12] Christensen, M.C. and Kaufmann, H. H. (1969). Grain storage. The Role of fungi in Quality Loss. University of Minnesota Press. Minneapolis, pp: 153.

[13] Christensen, M. C. and Meronuck, R. A. (1986). Quality maintenance in stored grains & seeds. Library of Congress, Univ. of Minnesota Press. Minneapolis. 138 pp.

[14] Cook, H. A. (1962). Barley and Malt. 1. Biology, Biochemistry and Technology. Academic Press, NewYork and London. 465 pp.

[15] Cook, R. J. and Vesth, R. J. (1991). Wheat Health Management. American Phytopathological Society, St. Paul. MN.

[16] Cretnin, Z. and Pepeljnjak, S. (1990). Ochratoxigenicity of A. ochraceus strains from nephropatic and non-nephropatic a areas in yugoshvia. Mycopathologia, 110:93-99.

[17] Fahmy, T.A. (1960). Fumigation of rice against insect pests and its effect on the quality of the grain. M.Sc. Thesis, Cairo University.

[18] Flannigan, B. (1978). Primary contamination of barley and wheat grain by storage fungi. Trans. Brit. Mycol. Soc., 71(1): 37-42.

[19] Hansen, H. N. (1926). A simple method of obtaining single spore culture, Science, 64: 384, 1659.

[20] Harrington, J.F. (1972). Seed stoage and longevity pp. 142-245. In Seed Biology: Kozolowski., T.T. (ed). Acad Press, London.
[21] Hill, A. R. and Lacey, J. (1983). Factors determining the microflora of stored barley grains. Ann. of Applied Biology, 102: 467-487.
[22] Hill, A. R., Lacey, J. and Reynolds, P. J. (1983). Storage of barley grain I iron age type underground pits. J. Stored Prod Res., 19(4): 163-171.
[23] Horn, S.W.R.L. Greene and J.D. Dorner (2000). Inhibition of aflatotin B, production by Aspergillusparasiticus using non-aflatoxigenic strains: Biological Control, 17: 147-154.
[24] ISTA (International Seed Testing Association) (1966). International Rules Health Testing Proc. International Seed Test Assoc., 31, 1-152.
[25] Jorgensen, J. (1970). Changes in the fungus flora on barley seed stored with a high moisture content. Tidsskr. PLA, 74(3): 425-432.[c.f. Rev. Pl. Path., 50(4): 210, 1971].
[26] Lacey, J. and Magan, N. (1991). Fungi in cereal grains. Their occurrence, water and temperature relationships. Cereal Chem., 26(5): 77-118.
[27] Lee, W. S. (1986). Studies of the mycoflora of barley during storage. Ph. D. Thesis, Massey University, New Zealand.
[28] Lutey, R. W. and Christensen, C. M. (1963). Influence of moisture content, temperature and length of storage upon survival of fungi in barley kernels. Phtiopatholog., 53: 713-717.
[29] Lynch, B.T., R.L. Glass and W. F. Geddes, (1962). Grain storage XXXII. Quantitative changes occurring in the sugars of wheat deteriorating in the presence and absence of molds. Cereal Chem., 39 (3): 256-262.
[30] Makun, H.A., T.A. gbodi; A.S. Tijani; A. Abal and G. U. Kadiri (2007). Toxicologic screening of fungi isolated from millet during the raing and dry hamttan seasons in Niger states, Nigeria. African J. Biotechnology, 6: 034-049.
[31] McGimpsey, C. H. and Malone, J. P. (1981). The fungal flora of stored undried barley grain with special reference to harmful organisms. Record of Agric. Res., 29: 99-102.
[32] Meronuck, A. R. (1983). Good grains storage. Agricultural Extension Sera. R.vice. Univ. of Minnesota. AG-Fo-0564. P. 1-8.
[33] Meronuck, (1987). The significance of fungi in cereal grains. Pl. Dis., 71(3): 287-290.
[34] Mills, J. T. (1990). Mycotoxins and toxigenic fungi on cereal grains in Western Canada, Can. J. Physiol., 68: 982-286.
[35] Murugan, S., Anand, R., Uma, P., Vidya, N. and Rajesh, K.A. (2007). Efficacy of Euphorbia pulcherrima on aflatxin producing fungi (Aspergillusflavus and A. parasiticus). African J. Biotechnology, 6(6): 718-719.
[36] Neergaard, P. (1977). Seed Pathology. The Mac-Millan Press, LTD. 1187 pp.
[37] Papavizes, G. C. and Christensen, C. M. (1960). Grain storage studies, effevt of invasion by individual species development of discolored germs in wheat. Cereal Chem., 37: 197-203.
[38] Pardo, E., Marin, S., Sanchis, V. and Ramos, A. J. (2004). Predication of fungal growth and ochratoxin A production by Aspergillusochraceus on irradiated barley grains as influeneed by temperature and water activity. International J. Food Microbiology, 95:79-88.

[39] Pomeranz, Y. (1976). Cereal grains handling system. Adances in Cereal Science and Techology: Am. Ass. Cereal Chem. St. Paul, USA, 217 pp.
[40] Prased, T. and Pathak, S. S. (1987). Impact of various storage systems on biodeterioration of cereal. Indian Phytopath., 40(1): 39-46.
[41] Qasem, S. A. and Christenesn, C. M. (1958). Influence of moisture content, temperature and time on the deterioration of stored corn by fungi. Phytopathology, 48: 544-549.
[42] Rasmusson, C. D. (1985). Barley. 2. Nutritional quality of barley American Society of Agronomy, Crop Science Society of America,. Soil Science Society of America, Publishers Madison, USA, 582 pp.
[43] Rauber, R and Jsselstein, J. (1985). Studies on thermosensitivity and water-sensitivity during germination of freshly ripened winter barley (Hordemvulgare). Angew Bot., 59(3-4): 267-277.
[44] Razzaghi-Abyaneh, M. Shams-Ghahfarokhi, M. yoshinari, T. Mohammed-Bagher, R. Jaimand, K. Nag a sawa, H. and Sakuda, S. (2008). Inhibitory effects of satuejahortensis L essential oil on growth and of latoxin of food Microbiology production by Aspergillusparasiticus. International J. 123: 228-233
[45] Riker, A. J. and Riker, R. S. (936). Introduction to research on plant Diseases. John. S. Swift Co, Inc. Sta. lovis Chicago, New York.
[46] Rosa, C. A. R., Keller, K. M., Keller, L. A. M., Gonzales Pereyra, M. L., Pereyra, C. M., Dalcero, A. M., Cavaglieri, L. R. and Lopez, C. W. G. (2009). Mycological survey and ochratoxin A natural comtamination of wine feedstuffe in Riode Janeiro State, Brazil. Toxican, 53: 83-288.
[47] Salgado, J. M. and Carvalho, P. C. T. (1980). Toxigenic fungi associated with grains. I. Survey of micoflora associated with corn, wheat and rice. Revista-de-Microbiologia, 11(2): 60-63. (c.f. Rev. Pl. Path., 60(8): 485, 1981).
[48] Salontai, A., Suciu, T., Musat, D. and Henegariu, O. (1989). Chemical Storage and conservation of cereal seeds. BuletinulInstitutului Agronomic, Cluj-Napoca, Agricultura, 41: 61-67. (c.f. Rev. Pl. Path., 68(1): 19, 1989).
[49] SAS (2006): SAS/STAT . Guide for personal Computer: SAS, Inst. Cary. N. C., USA.
[50] Sauer, B. D., Meronuck R. A. and Christensen, C. M. (1992). Storage of cereal grains and their products. 7. Microflora. Am. Ass. Cereal Chem. Inc. St. Paul, MN., USA, 813 pp.
[51] Sejiny, M. J., Tawfik, A. K. and El-shaieb, K. M. (1984). Studies on mycoflora of cereal grains in the southern west region of Saudi Arabia. I. Fungi associated with some cereal grains at post-harvest and during storage. Ann. Agric. Sci., Moshtohor, 22(1): 281-297.
[52] Simon, C. and Sivasithamparam, M. (1988). Interactions among G: graminisvartritici, Trichodermakoningii and soit bacteria. Can. J. Microbiol., 34: 871-876.
[53] Singh, S.N. and Chand L.(1993). Inhibition of aflatoxin production by garlic and sodium bicarbonate. Crop Res., 6:149-154.
[54] Sinha, R. N. and Wallace, H. A. H. (1977). Storage stability of farm-stored rapeseed and barley. Canadian J. of Plant Sci., 57(2): 351-365.
[55] Stroshine, R., Tuite, J., Foster, H. G. and Baker, K. (1984). Self-study guide for grain during storage. Purdue University, USA, 131 pp.

[56] Sulaiman, E. D. and Husain, S. S. (1984). Pathogenicity and effect on germination caused by Aspergillus and Penicillium on wheat, rice, barley and corn. Pakis. J. Sci. Ind. Res., 27(6) 359-362.
[57] Tsuruta, D. (1974). Infestation of domestic cereals by microorganisms. I. Fungi on wheat and barley. Rep. of the Nat. Food Res. Inc., 29: 16-20.
[58] Tuite, J. F. and Christensen, C. M. (1952). Fungi important in storage of barley. Phytopathology, 42: 476.
[59] Tuite, J. F. and Christensen, C. M. (1955). Grain storage studies XVI. Influnce of storage condition upon the fungus flora of barley seed. Cereal Chem., 32(1): 1-11.
[60] Yaqub, F. and Shahzad, S. (2005). In vitro evaluation of microbial antagonists against Sclerotiumrolfsii. Pak. J. Bot., 37(4): 1033-1036.
[61] Zeleny, L. (1954). Chemical, Physical and nutritive changes during storage P. 46-76 in 3.A. Anderson and A.W.Alcom (eds), storage of cereal grains and their products. AM. Assoc: Creal Chem. St. Pual. Minn.
[62] Zhou, T. and Releder, R. D. (1990). Selection of Epicoccumpurpurascens and improved biocontrol of Sclerotiniasclerotiorum. Can. J. Microbial., 36: 754-759.