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Studies on Seed Mycoflora of Wheat (*Triticum aestivum* L.) Treated with Potassium Nitrate and its Effect on Germination During Storage

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ABSTRACT

The main aim of the present study was to enumerate the fungal species and their effect on germination associated with wheat seeds. Seeds of two cultivars *viz.*, Kundan and HUW-234 of wheat (*Triticum aestivum* L.) were collected after harvesting from agriculture farm, Banaras Hindu University, Varanasi. These seeds were treated with potassium nitrate and examine for seed mycoflora by agar plate method and blotter method. Total sixteen fungal species were isolated from test cultivars by the standard techniques. Fungi isolated and identified were *Alternaria alternata, Alternaria solani, Aspergillus candidus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Curvularia lunata, Fusarium roseum, Fusarium semitectum, Penicillium citrinum, Penicillium rubrum, Rhizopus stolonifer, Trichoderma harzianum,* Dark sterile mycelium and White sterile mycelium. During isolation, the blotter method yielded the higher number of fungi as compared to agar plate method. Germination of infested wheat seeds was determined by three methods *viz.*, blotter method, multi-pot tray method and plastic pot method. Germination was decreased during storage period because fresh seeds showed better germination percent i.e., from 95 to 100% than stored seeds. Nitrate treated seeds show better germination percent than untreated seeds of both cultivars.

Key words: Cultivars, seed hardening, germination, agar plate method, blotter method

INTRODUCTION

Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Numerous examples exist in agriculture literature for the international spread of plant diseases as a result of the importation of seeds that were infected or contaminated with pathogens (Agarwal and Sinclair, 1996).

Seed-borne diseases have been found to affect the growth and productivity of crop plants (Kubiak and Korbas, 1999; Weber *et al.*, 2001; Dawson and Bateman, 2001). A seedborne pathogen present externally or internally or associated with the seed as contaminant, may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Khanzada *et al.*, 2002; Bateman and Kwasna, 1999).

Wheat is one of the first cereals known to have been domesticated and wheat's ability to self-pollinate greatly facilitated the selection of many distinct domesticated varieties. Wheat is one of the main staple foods of man and is grown in almost all the temperate and subtropical regions of the world. Seed-borne mycoflora of wheat reported recently included *Alternaria alternata, Drechslera sorokiniana, Fusarium moniliforme, F. avenaceum, F. graminearum, F. nivale, F. culmorum, F. equiseti, F. sporotirchioides, Cladosporium herbarum, Stemphylium botryosum* (Nirenberg *et al*., 1994; Glazek, 1997; Mirza and Qureshi, 1978). The effects of such fungi on the seedlings growth include poor germination and less vigorous seedlings.

Seed health plays an important role for successful cultivation and yield exploitation of a crop species. Among various factors that affect seed health, the most important are the seed borne fungi that not only lower seed germination but also reduce seed vigor resulting in low yield. Healthy seed plays an important role not only for successful cultivation but also for increasing yield of crop (Rajput *et al*., 2005). Seed-borne pathogens of wheat are responsible to cause variation in plant morphology and also reducing yield upto 15-90% if untreated seeds are grown in the field (Wiese, 1984). Seed borne infection of fungal pathogens are important not only for its association with the seeds which cause germination failure and/or causing disease to the newly emerged seedlings or growing plants but also contaminate the soil by establishing its inocula permanently (Hasan *et al*., 2005).

The main objective of this study is to see the effect of potassium nitrate on the seed mycoflora during storage and also see the effect of seed hardening on germination abiHty of both the cultivars of wheat.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi from April 2009 to March 2010.

In the present work an attempt has been made to study the seed mycoflora of two cultivars, *viz*., Kundan and HUW-234 of the wheat (*Triticum aestivum* L.).

Harvested seeds of the year 2009 were collected from Agricultural farm, Banaras Hindu University through plant breeder after Rabi season and stored in glass bottles covered with lid under laboratory condition up to one year at room temperature. The experimental data were recorded from fresh seeds as well as those stored, after every three months (*viz*., 90, 180, 270 and 360 days) of storage.

Another lot of seeds from both varieties (Kundan and HUW-234) were treated with potassium nitrate (seed hardening) for detailed study. For this, seeds were surface sterilized with 0.1% $HgCl_2$ for 1-2 min and washed thoroughly with distilled water and soaked for 16 h in KNO_3 solution (15 mM) in nitrate at temperature of 20±2°C. These seeds were dried back again to its original weight and used for further studies. Three rephcations of each treatment were prepared. Isolation of seed mycoflora from both untreated and potassium nitrate treated wheat seeds were done by agar plate and blotter method.

Agar plate technique: The seeds were externally sterilized by 0.1% mercuric chloride solution to 1 to 2 min then washed by sterilized distilled water (Habib *et al*., 2007). The isolated fungi were identified using light microscope after slides were stained lactophenol (Anonymous, 1994; Henselova and Hudecova, 2001; Gwary *et al*., 2006).

Blotter test: The blotter test (Limonard, 1966; Lantos *et al*., 2002) was used to isolate the fungal pathogens associated with the seeds during storage. The seeds were externally sterilized by 0.1% mercuric chloride solution to 1 to 2 min then washed by sterilized distilled water (Habib *et al*., 2007).

Identification of fungi: After incubation the growth characters as well as percentage of infection were recorded. In order to isolate these fungi into pure culture, Potato Dextrose Agar (PDA) was prepared and the fungi were inoculated onto the sterile PDA and incubated for 7 days at the end of which the fungi were identified based in their colour, spore morphology and mycelia growth using the light microscope (Begum *et al*., 2004; Chuku *et al*., 2007; Al-Sheikh, 2009). The fungi were identified by Thom and Raper (1945), Raper and Thom (1949), Booth (1971), Ellis (1971) and Barnett and Hunter (1972).

Determination of seed germination (%) of fresh and stored seed

Blotter method: Three pieces of blotting paper were placed in fold in each Petri dish 9 cm diameter and incubated at 25±2°C. Three replicates were prepared. The percent germination of wheat seed was calculated after one week (De Tempe, 1953).

Multi-pot tray method: Inverted egg trays were used as the multi-pot trays to test the germination of *Triticum aestivum* seeds in glass house. The small pots were filled with the pre-sterilized (95°C for 30 min in autoclave) uniform soil mixture containing 4 parts peat with essential amount of fertilizer and seeds were sown in them. The trays were covered with a polyethylene envelope so as to reduce water loss through evaporation (Khare, 1996).

Plastic pot method: In this method plastic pots were used to test for determination of percent germination of *Triticum aestivum* seeds in glass house. The plastic pot filled with the pre-sterilized (95°C for 30 min in autoclave) uniform soil mixture containing 4 parts peat with essential amount of fertilizer and seeds sown in them the pots were covered with a polypropylene envelope so as to reduce water loss through evaporation. The germination was counted 5 to 7 days after sowing.

The first count for germinated seeds was taken 5 days and the second 7 days after plating, respectively.

RESULTS

A total of 16 fungal species *viz*., *Alternaria alternata, Alternaria solani, Aspergillus candidus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Curvularia lunata, Fusarium roseum, Fusarium semitectum, Penicillium citrinum, Penicillium rubrum, Rhizopus stolonifer, Trichoderma harzianum,* Dark sterile mycelium and White sterile mycelium were isolated from wheat seed during different periods of storage from April 2009 to March 2010 (Table 1, 2).

The dominant field fungi recorded from fresh seeds was *Alternaria alternata, A. solani, Rhizopus stolonifer, Curvularia lunata, Trichoderma viride, Fusarium oxysporum. Penicillium* spp., most of these field fungi were replaced by storage fungi *viz*., *Aspergillus niger, A. flavus, A. terreus, Penicillium citrinum, Tricoderma viride*. It has been observed that the field fungi decreased along with increase in storage time. It is evident from observation that maximum fungi were recorded in rainy season and summer season and lesser number of species was recorded in winter season.

Table 1: Fungi isolated from seeds of *Triticum aestivum* by Agar Plate Method during different period of storage

	Seed treated with Nitrate										Control									
	Fresh seeds		90 days		180 days		270 days		360 days		Fresh seeds		90 days		180 days		270 days		360 days	
Fungal species	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Alternaria alternata	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Alternaria solani	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus flavus	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Aspergillus terreus	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-
Aspergillus fumigatus	+	-	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+
Aspergillus candidus	-	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-
Curvularia lunata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium roseum	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Fusarium semitectum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium citrinum	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillium rubrum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
Rhizopus stolonifer	+	+	-	-	-	-	+	-	+	-	+	+	-	-	+	+	+	+	+	+
Trichoderma spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+
Dark sterile mycelium	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
White sterile mycelium	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-	+	-	+	-

A: Variety-A (Kundan) B: Variety-B (HUW-234). +: Present. -: Absent

Table 2: Fungi isolated from seeds of *Triticum aestivum* by Blotter Method during different period of storage

	Seed treated with Nitrate										Control									
	Fresh seeds		90 days		180 days		270 days		360 days		Fresh seeds		90 days		180 days		270 days		360 days	
Fungal species	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Alternaria alternata	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+
Alternaria solani	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus flavus	+	-	+	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+
Aspergillus terreus	-	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
Aspergillus fumigatus	+	-	+	-	+	-	-	-	+	-	+	-	+	-	+	+	+	-	+	-
Aspergillus candidus	+	-	+	-	+	-	+	-	+	-	+	+	+	+	+	-	-	-	+	+
Curvularia lunata	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium roseum	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium semitectum	-	+	+	+	-	-	-	+	-	+	+	+	+	-	+	+	+	-	+	+
Penicillium citrinum	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Penicillium rubrum	-	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-
Rhizopus stolonifer	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-
Trichoderma spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
Dark sterile mycelium	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
White sterile mycelium	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-

A: Variety-A (Kundan) B = Variety-B (HUW-234). +: Present. -: Absent

As the observations depicted in total reveal, more number of fungi were isolated by blotter method as compared to agar plate method. This may be due to the reason that some of the slow

growing fungi and weak competitors could not grow in the culture plates in competitions to the fast growing fungi and the selective nature of the culture medium which might not have favoured the growth of such species. Some fungi *viz.*, *Helminthosporium* and *Fusarium semitectum* were observed only by blotter method.

Data presented in Table 3 reveals that in blotter method there is decrease in percent germination of wheat seeds along with the storage period. In fresh wheat seeds no effect shown on the control and nitrated treated test cultivars. Maximum percent germination was shown by the fresh seeds of cultivar Kundan i.e., 98% both in control and after nitrate treatment and minimum percent germination were shown by 360 days old untreated seeds of cultivar Kundan i.e., 79%.

Data presented in Table 4 reveals that in multi-pot trays, both nitrate treated wheat cultivars (Kundan and HUW-234) shown more germination than untreated seeds during storage. Maximum percent germination was shown by fresh nitrate treated seeds of cultivar Kundan i.e., 100% followed by the control of fresh seeds of both the cultivars and minimum germination was shown by 360 days old untreated seeds of cultivars HUW-234 i.e., 79%.

Data presented in Table 5 reveals that in plastic pots, the percentage germination of nitrate treated cultivar Kundan increase than untreated seeds of Kundan. The percentage seed germination of treated cultivar Kundan was more than treated cultivar HUW-234. Maximum percent germination was shown by the fresh seeds of cultivar Kundan under untreated and treated conditions i.e., 97% followed by the fresh seeds of cultivar HUW-234. Minimum percent germination was shown by 270 days old untreated seeds of cultivar HUW-234 i.e., 79%.

Table 3: Percent germination of seeds of *Triticum aestivum* during different period of storage by blotter method

	Seed germination (%)			
	(Cv. Kundan)		(Cv. HUW 234)	
Storage period (day)	Control	Nitrate treated	Control	Nitrate treated
Zero (Fresh seeds)	98	98	97	97
90	93	94	92	93
180	88	90	86	89
270	83	87	81	85
360	79	81	78	80

Table 4: Percent germination of seeds of *Triticum aestivum* seeds during different period of storage by Multi-pot tray method

	Seed germination (%)			
	(Cv. Kundan)		(Cv. HUW 234)	
Storage period (days)	Control	Nitrate treated	Control	Nitrate treated
Zero (Fresh seed)	99	100	99	99
90	96	99	95	97
180	94	97	90	93
270	88	93	83	91
360	84	88	79	83

Table 5: Percent germination of *Triticum aestivum* seeds during different period of storage by plastic pot method

	Seed germination (%)			
	(Cv. kundan)		(Cv. HUW 234)	
Storage period (day)	Control	Nitrate treated	Control	Nitrate treated
Zero (Fresh seed)	97	97	95	95
90	92	92	91	93
180	88	84	83	86
270	85	81	79	83
360	82	80	78	80

DISCUSSION

The fungi isolated from stored wheat seeds were the main cause of deterioration of seeds during storage (Worang *et al*., 2008; Dharmaputra *et al*., 2009). Surface sterilization also has the advantage of minimizing competition among fungi on the seed (Kaur, 2010). Seed surface disinfection with $HgCl_2$ usually suppresses the growth of saprophytic and other superficial fast growing fungi (Limonard, 1968; Bhutta, 1988). It was also observed by Ramakrishna *et al*. (1991) that surface sterilization with 0.1 or 0.2% (w/v) $HgCl_2$ for 3 min significantly decreased *Alternaria alternata, Fusarium* spp. and *Epicoccum purpurascens* but Niaz and Dawar (2009) observed that surface disinfection of seed with 1% $Na(OCl)_2$ reduced the incidence of *Aspergillus* spp., *Chaetomium* spp., *Cladosporium* spp., *Rhizopus* spp., *Cephalosporium* spp. Reduction of frequency rate of fungi from sterilized sunflower seeds was also found by Sharfun-Nahar *et al*. (2005) and Bhutta *et al*. (1998). Of the two methods used, the blotter method yielded the highest number of fungi as compared to agar plate method. Singh (1999) also reported it, that more fungi were isolated by blotter technique than the agar plate method. This may be show to growing fungi and weak competitors could not grow in cultivar plate in competition. Some fungi were observed only on the agar plate technique *viz*., *Alternaria solani*. It has been observed that the field fungi decreased along with increase in storage time. It is evident from observation that maximum fungi were recorded in rainy season and summer season and lesser number of species was recorded in winter season. Similar observation were also recorded by Lal and Kapoor (1979) while studied the succession of fungi on wheat and maize seeds during storage, reported the general decline of the field fungi is due to development of storage fungi as under the ecological condition prevailing during storage the latter can thrive better. Amongst the species of *Aspergillus niger, A. flavus* and *A. terreus* were the most dominant. Decreasing in number of fungal species during storage has also been reported by Reddy and Reddy (1983), Vijaylakshimi and Rao (1985), Paul and Mishra (1992) and Singh (1999). The dominant fungi and fungal growth depend on period and environmental conditions (Al-Yahya, 1999; Krasauskas *et al*., 2005). In both varieties the hardened seed showed better germination than control. The effects of seed treatment with chemicals on germination were reported by Sharma and Bose (2006).

CONCLUSION

From the present study it was concluded that wheat seeds are constantly subjected to deterioration which implies an irreversible degenerative change in the quality of seeds and also reduced their germination ability.

REFERENCES

Agarwal, V.K. and J.B. Sinclair, 1996. Principles of Pathology. 2nd Edn., CRC Press Inc., Boca Raton, FL., pp: 539.

Al-Sheikh, H., 2009. Date-Palm fruit spoilage and seed-borne fungi of Saudi Arabia. Res. J. Microbiol., 4: 208-213.

Al-Yahya, S.A., 1999. Change of fungal infection during wheat storage at different conditions. Arab Univ. J. Agric. Sci., 7: 531-545.

Anonymous, 1994. The microbiology of water 1994 part 1: Drinking water. Report on Public Health and Medical Subjects, No. 71. Methods for the Examination of Waters and Associated Materials. HMSO, London.

Barnett, H.L. and B.B. Hunter, 1972. Illustrated Genera of Imperfect Fungi. 3rd Edn., Burgess Publishing Company, Minneapolis, pp: 331.

Bateman, G.L. and H. Kwasna, 1999. Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. Applied Soil Ecol., 13: 271-282.

Begum, N., K.Z. Alvi, M.I. Haque, M.U. Raja and S. Chohan, 2004. Evaluation of mycoflora associated with pea seeds and some control measures. Plant Pathol. J., 3: 48-51.

Bhutta, A.R., 1988. Comparison of cotton seed health testing method and their economics. Pak. Cotton, 32: 146-153.

Bhutta, A.R., M.H. Rahber-Bhatti, G.R. Solangi, I. Ahmed and M.H. Rehman, 1998. Sunflower production and pathological problems. Sci. Technol. Dev., 2: 51-55.

Booth, C., 1971. The Genus *Fusarium*. 1st Edn., Commonwealth Mycological Institute, Kew, Surrey, England, pp: 237.

Chuku, E.C., O.K. Ogbalu and J.A. Osakwe, 2007. Fungi of coconut (*Cocos nucifera* L.)-their deteriorative ability, quality stability and the role of the fungus-eating insects. J. Applied Sci., 7: 3106-3110.

Dawson, W.A.J.M. and G.L. Bateman, 2001. Fungal communities on roots of wheat and barley and effects of seed treatments containing fluquinconazole applied to control take-all. Plant Pathol., 50: 75-82.

De Tempe, J., 1953. The blotter method of seed health testing. Proc. Int. Seed Test. Assoc., 21: 133-151.

Dharmaputra, O.S., R.L. Worang, R. Syarief and Miftahudin, 2009. The quality of physic nut (*Jatropha curcas*) seeds affected by water activity and duration of storage. Microbiology, 3: 139-145.

Ellis, M.B., 1971. Dematiaceous Hyphomycetes. 1st Edn., Commonwealth Mycological Institute, Kew, Surrey, UK., pp: 608.

Glazek, M., 1997. Mycoflora of winter wheat seeds harvested from flooded commercial fields in South-Western Poland in 1997. Plant Prot. Inst. Poznaniu, 29: 44-153.

Gwary, D.M., D.M. Mailafiya and T.J. Jibrin, 2006. Survival of *Colletotrichum sublineolum* and other seed-borne fungi in sorghum seeds after twenty months of storage. Int. J. Agric. Biol., 8: 676-679.

Habib, A., S.T. Sahi, M.U. Ghazanfar and S. Ali, 2007. Location of seed-borne mycoflora of eggplant (*Solanum melongena* L.) in different seed components and impact on seed germinability. Int. J. Agric. Biol., 9: 514-516.

Hasan, M.M., S.P. Chowdhury, S. Alam, B. Hossain and M.S. Alam, 2005. Antifungal effects of plant extracts on seed-borne fungi of wheat seed regarding seed germination, Seedling health and vigour index. Pak. J. Biol. Sci., 8: 1284-1289.

Henselova, M. and D. Hudecov, 2001. Differences in the microflora of scarified and unscarified seeds of *Karwinskia humboldtiana* (Rhamnaceae). Folia Microbiol., 46: 543-548.

Kaur, B., 2010. Development and evaluation of methods for the detection of seed borne fungi in rice. Int. J. Educ. Administration, 2: 123-130.

Khanzada, K.A., M.A. Rajput, G.S. Shah, A.M. Lodhi and F. Mehboob, 2002. Effect of seed dressing fungicides for the control of seedborne mycoflora of wheat. Asian J. Plant Sci., 1: 441-444.

Khare, M.N., 1996. Methods to test seeds for associated fungi. Indian Phytopathol., 49: 323-323.

Krasauskas, A., A. Steponaviciene, M. Railiene, A. Lugauskas, A. Raila and V. Raudoniene, 2005. Impact of environmental conditions on the spread of micromycetes in grain during its harvesting and storage. Bot. Lithuanica, 11: 101-109.

Kubiak, K. and M. Korbas, 1999. Occurrence of fungal diseases on selected winter wheat cultivars. Postepy Ochronie Roslin, 39: 801-804.

Lal, S.P. and J.N. Kapoor, 1979. Succession of fungi in wheat and maize during storage. Indian Phytopathol., 32: 101-104.

Lantos, F., J. Lantos and E. Lantos, 2002. An unusual twist to the blotter test: The lantos method. Practicing Oil Analysis, pp: 7. http://www.v-eight.com/tech_forum/viewtopic.php?f=7&t=21.

Limonard, T., 1966. A modified blotter test for seed health. Eur. J. Plant Pathol., 72: 319-321.

Limonard, T., 1968. Ecological Aspects of Seed Health Testing. International Seed Testing Association, Wageningen, Netherlands, pp: 167.

Mirza, J.H. and M.S.A. Qureshi, 1978. Fungi of Pakistan. University of Agriculture, Faisalabad, Pakistan.

Niaz, I. and S. Dawar, 2009. Detection of seed borne mycoflora in maize (*Zea mays* L.). Pak. J. Bot., 41: 443-451.

Nirenberg, H., H. Schmitz-Elsherif and C.I. Kling, 1994. Occurrence of *Fusaria* and some blackening moulds on durum wheat in Germany. 1. Incidence of *Fusarium* species. Pflanzenkrankheiten Pflanzenschuntz, 101: 449-459.

Paul, M.C. and R.R. Mishra, 1992. Studied on seed mycoflora of maize. I. Seasonal variation in mycoflora. Crop. Res., 5: 225-232.

Rajput, M.A., M.A. Pathan, A.M. Lodhi, G.S. Shah and K.A. Khanzada, 2005. Studies on seed-borne fungi of wheat in Sindh province and their effect on seed germination. Pak. J. Bot., 37: 181-185.

Ramakrishna, N., J. Lacey and J.E. Smith, 1991. Effect of surface sterilization, fumigation and γ irradiation on the microflora and germination of barley seeds. Int. J. Food Microbiol., 13: 47-54.

Raper, K.B. and C. Thom, 1949. A Manual of the *Penicillia*. 1st Edn., Williams and Wilkins Co., Baltimore, Maryland.

Reddy, A.S. and S.M. Reddy, 1983. Influnce of seed moisture on fungal succesion on seeds of sesamum *Sesamum indicum*. Seed Res., 10: 120-124.

Sharfun-Nahar, M. Mushtaq and M.H. Hashmi, 2005. Seed-borne mycoflora of sunflower (*Heliathus annuus* L.). Pak. J. Bot., 37: 451-457.

Sharma, M.K. and B. Bose, 2006. Effect of seed hardening with nitrate salts on seedling emergence, plant growth and nitrate assimilation of wheat (*Triticum aestivum* L.). Physiol. Mol. Biol. Plants, 12: 173-176.

Singh, G., 1999. Studies on seed borne fungi of mungbean (*Vigna radiata* L.) during storage and their control. M.Sc. Thesis, Banaras Hindu University, Varanasi, UP.

Thom, C. and K. Raper, 1945. A Manual of the *Aspergilli*. 1st Edn., Williams and Wilkins Co., Baltimoreg, USA.

Vijaylakshimi, M. and A.S. Rao, 1985. Fungal infection of sunflower seeds under different conditions of storage. Indian Phytopathol., 38: 315-318.

Weber, R., B. Hrynczuk, B. Runowska-Hrynczuk and W. Kita, 2001. Influence of the mode of tillage on diseases of culm base in some winter wheat varieties, oats and spring wheat. J. Phytopathol., 149: 185-188.

Wiese, M.V., 1984. Compendium of Wheat Diseases. 3rd Edn., American Phytopathology Society, UK., pp: 106.

Worang, R.L., O.S. Dharmaputra, R. Syarief and Miftahudin, 2008. The quality of physic nut (*Jatropha curcas*) seeds packed in plastic material during storage. Biotropia, 15: 25-36.