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Ecological Biodiversity Measurement of Seed Mycoflora Contamination of Freshly Harvested in Maize Growing Zone-II

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Maize is considered third most important cereal crops in the world. In our study the seed mycoflora of freshly harvested maize of zone-II were isolated by Agar plate method (APM) and Blotter plate method (BPM). A total of 9 genera i.e. *Aspergillus flavus*, *A. niger*, *Bipolaris maydis*, *Curvularia lunata*, *Fusarium verticilioides, Penicillium notatum, P. expensum, Rhizopus stolonifer,* and *Rhizoctonia solani* were isolated by standard Agar plate method and 10 fungal genera, i.e. *Alternaria alternata, Aspergillus flavus*, *A. niger*, *Bipolaris maydis*, *Curvularia lunata*, *Fusarium verticilioides, Macrophomina phaseolina, Penicillium notatum, Rhizopus stolonifer,* and *Rhizoctonia solani* by blotter plate method. On the basis of density, frequency and abundance, *Aspergillus flavus, A. niger* and *Rhizopus stolonifer* were found as dominate and taken for detail study. The seed lot of this zone is three categories i.e. Original (OS), Partial discolour (PDS) and Discolour seed (DS). Maximum important value index (IVI), Simpson index of dominance (D), Shannon-Weaver index of diversity (H) and Evenness (E) of *Aspergillus flavus* OS (86.657%, 0.0834, 0.359, 0.184), PDS (63.827%, 0.0453, 0.329, 0.150) and DS (83.467%, 0.0774, 0.356, 0.183) were contributed. In Blotter plate method, highest density of *A. niger* OS (5.850), *A. flavus* PDS (4.500), DS (5.225) were recorded. Maximum frequency showed by *A. flavus* (100.000%) in all categories. The abundance of *A. flavus* OS (0.350), PDS (0.342), DS (0.407) were recorded. Relative density maximum recorded in *A. niger* OS (26.401%), *A. flavus* PDS (19.268%) and DS (26.381%). Relative frequency (RF) and relative abundance (RA) highest were found in *A. flavus* OS (24.691%, 35.009%), PDS (23.256%, 34.221%) and DS (23.810%, 40.661%). Maximum IVI, Simpson index of dominance, Shannon-Weaver index of diversity and evenness contributed *A. flavus* OS (80.912%, 0.0727, 0.353, 0.170), PDS (76.744%, 0.0654, 0.349, 0.159) and DS (90.852 %, 0.0917, 0.362, 0.174). These species are some of the common on the maize during storage and spoil the grains.

Keywords: *Aspergillus flavus, A. niger, Zea mays,* Simpson index of dominance and Shannon-Weaver index of diversity.

Maize (*Zea mays* L.) is a staple food for approximately 400 million people in the worldwide for processed food and feed[1]. In India, maize ranks fifth in total area and third in total production and productivity. It is susceptible to a numerous fungal species that cause ear and kernel rots including, *Aspergillus*, *Fusarium verticillioides*, *F. proliferatum, F. subglutinans*, *Gibberella zeae Penicillium*, *Macrophomina phaseolina*, *Diplodia*, *Nigrospora*, *Botryosphaeria*, *Cladosporium*, *Trichoderma*, *Rhizoctonia*, and *Rhizopus*[2, 3]. There has been continuous increase in the world population then consumption demand of corn to be increase in the demand from poultry and piggery sector used as a feed. In the presence of seed borne pathogens several types of

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abnormalities occur in the seeds. Such seeds are rejected by seed industries and for agricultural purposes. Since the fact endeavor has been made to study the maize seed mycoflora and their cheaper eco-friendly management. Seed borne mycoflora is one of the major components reducing the maize yield. Mycoflora associated with seeds both internally and externally are responsible for seed major step is to use disease free and certified seed[4, 5]. Fungal species are related to corn mostly belong to *Apergillus* spp *Fusarium* spp. and *Penicillium* spp. There are many reports that indicate these fungal species produce dangerous mycotoxin which can be harmful for human health and animals[6, 7, 8]. Usually, fungal species diversity is one of the most important indices used to evaluation of an ecosystem. A large value of Shannon-Wiener Index (H) has showed a rich ecosystem with high species diversity and low value (H') will have a low species diversity[9, 10].The present study was aimed at ecological biodiversity measurement of Seed mycoflora contamination of freshly harvested in maize growing zone-II.

MATERIAL AND METHODS

The maize growing area in to three zones, i.e. zone-I, (Almora, Kullu, Bilaspur, Daulakauna Kangra and Saharanpur) zone-II (New Delhi, Karnal, Pantnagar and Ludhiana) and zone-III (Varanasi and Begusarai). In this study, four maize seed samples were taken from maize growing Zone-II. The collected seed samples of each maize variety will be critically examined and grouped into three categories with the help of hand lens i.e. original seed (OS), partially discoloured seed (PDS) and discoloured seed (DS). Myco-flora detected on maize seed by Agar plate method-APM[11] and Blotter plate method-BPM[12]. One hundred seeds of each category of different varieties untreated will be place in a plastic Petri plates (90 mm dia.) lined with two layers of blotting papers moistened with distilled water for studying the association of different myco-flora with maize seeds. Ten seeds will be placed in each Petri plates equidistantly (pattern-1-3-6). The Petri plates will be incubated at 25 ± 1°C for five days and the seeds will be examined regularly for the presence of different fungi. There will be two replications each having 50 seeds. Incubated seeds will be examined visually and under Stereo-zoom microscope for the associated myco-flora. The associated fungi were isolated on PDA for further identification. Same method applied in Agar plate method also. The seed mycoflora were identified with the help of literature[13-20].

Based on the individuals fungi recorded in the distinct seed samples were analysed for density, frequency, abundance, relative density, relative frequency, relative abundance, importance value index, Simpson index of Dominance, Shannon- Weaver Index of Diversity and evenness. The importance value index of seed sample was determined as the sum of relative frequency, relative density and relative dominance[21].

Density is calculated by the equation:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all Petri plate}}{\text{Total number of Petri plate studied}}$$

Frequency (%) is calculated by the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of Petri plate in which the species occurred} \times 100}{\text{Total number of Petri plate studied}}$$

Abundance- It is the study of the number of individuals of different species in the community per unit area. It is represented by the equation:

$$\text{Abundance} = \frac{\text{Total number of individuals of a species in all Petri plate}}{\text{Total number of Petri plate in which the species occurred}}$$

Relative density, relative frequency and relative abundance was calculated as:

$$\text{Relative density} = \frac{\text{Number of individuals of a species} \times 100}{\text{Number of Petri plate studied}}$$

$$\text{Relative frequency} = \frac{\text{Number of occurrence of the species} \times 100}{\text{Number of occurrence of all the species}}$$

$$\text{Relative abundance} = \frac{\text{Total basal area of the species} \times 100}{\text{Total Petri plate of all the species}}$$

Importance Value Index (IVI)- It was calculated by equation[22]-

IVI = Relative frequency + Relative density + Relative dominance,

The maximum importance value for any one genus is 300 (100 + 100 + 100). It is useful, as it

provides an overall picture of the density, frequency and cover of a genus in relation to community.

Simpson's Dominance Index (D) - The Simpson's index (D) is calculated using the following equation[23]:

$$D = \frac{\sum_{i=1}^{s} n_i(n_i - 1)}{n(n-1)}$$

Where 'ni' is the proportion of individuals of the ith species in the community. Simpson's index gives relatively little weight to the rare species and more weight to the common species. It weighs towards the abundance of the most common species. It ranges in value from 0 (low diversity) to a maximum of (1-1/s), where s is the number of species. In nature the value of d ranges between 0 and 1. With this, index 0 represents infinite diversity and 1, no diversity. The bigger the (D) value, the smaller the diversity.

Shannon-Wiener Index (H)- This is a widely used method of calculating biotic diversity in aquatic and terrestrial ecosystems and is expressed as SWI [24]:

$$H' = \sum_{i=1}^{s} \frac{n_i}{n} \ln \frac{n_i}{n}$$

Where, H= index of species diversity s= number of species ni= proportion of total sample belonging to the ith species.

Evenness Index (E) - This is relative distribution of individuals among taxonomic groups within a community and is expressed[25] as:

$$E = H'/\log S$$

Where, H' = Shannon –Wiener diversity index, and log S= Natural log of the total number of species (S defined as **Species Richness**) recorded.

RESULTS AND DISCUSSION

Working seed samples were collected from zone-II (New Delhi, Karnal, Pantnagar and Ludhiana). In this study, four maize seed samples were taken from maize growing Zone II category.

A total of 9 genera were recorded within three seed categories through Agar plate me00thod. Association of *Aspergillus flavus*, *A. niger*, *Bipolaris maydis*, *Curvularia lunata*, *Fusarium verticilioides*, *Penicillium notatum*, *P. expensum*, *Rhizopus stolonifer*, and *Rhizoctonia solani* were observed (Table-1). Maize mycoflora was presented with 10 fungal genera, i.e. *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Bipolaris maydis*, *Curvularia lunata*, *Fusarium verticilioides*, *Macrophomina phaseolina*, *Penicillium notatum*, *Rhizopus stolonifer*, and *Rhizoctonia solani* by Blotter plate method (Table-2).

In Agar plate method, Highest density and relative density of *A. niger* OS (5.250, 26.960), and DS (5.267, 25.303) were recorded.

Density, frequency, abundance of *A. flavus* OS (4.875, 100.00, 0.356), PDS (4.800, 75.00, 0.258) and DS (4.925, 100.00, 0.348) were observed. Relative density, highest frequency, abundance by *A. flavus* OS (25.034, 25.974, 35.649), PDS (19.948, 18.072, 25.806) and DS (23.661, 25.000, 34.806) were recorded. Highest Important value index (IVI), Simpson index of dominance (D), Shannon-Weaver index of diversity (H) and evenness (E) of *A. flavus* OS (86.657%, 0.0834, 0.359, 0.184), PDS (63.827%, 0.0453, 0.329, 0.150) and DS (83.467%, 0.0774, 0.356, 0.183) were contributed.

Diversity of myco-flora in the study calculated using the Shannon-Weiner diversity index (H') showed values range OS (0.359-0.086), PDS (0.329-0.051) and DS (0.356-0.126). The values for Simpson index of dominance ranges were OS (0.0834-0.0005), PDS (0.0453-0.0001) and DS (0.0774-0.0015). Pielou's evenness index of myco-flora in OS, PDS and DS samples showed value ranges of 0.184-0.044, 0.150-0.023 and 0.183-0.065, respectively (Table -1).

In Blotter plate mathod, Highest density were recorded *A. niger* OS (5.850), *A. flavus* PDS (4.500), DS (5.225). Maximum frequency and abundance values *A. flavus* OS (100.000%, 0.350), PDS (100.000%, 0.342) and DS (100.000%, 0.407) were showed. Relative density maximum recorded in *A. niger* OS (26.401%), *A. flavus* PDS (19.268%) and DS (26.381%). Relative frequency and relative abundance highest in *A. flavus* OS (24.691%, 35.009), PDS (23.256%, 34.221) and DS (23.810, 40.661%) were intended. Maximum IVI, Simpson index of dominance, Shannon-Weaver index of diversity and evenness contributed *A. flavus* OS

Table 1. Biodiversity analysis of Seed mycoflora in maize by Agar plate method

		Zone II									
Ct	Species	Dn	F (In %)	Ab	RD (In %)	RF (In %)	RA (In %)	IVI (In %)	D=Pi*Pi	H=-{(pi) × ln(pi)}	E={H/ln(S)}
OS	*Aspergillus flavus*	4.875	100.000	0.356	25.034	25.974	35.649	86.657	0.0834	0.359	0.184
	Aspergillus niger	5.250	70.000	0.269	26.960	18.182	26.874	72.015	0.0576	0.343	0.176
	Rhizopus stolonifer	4.367	75.000	0.239	22.424	19.481	23.949	65.853	0.0482	0.333	0.171
	Penicillium expensum	1.300	25.000	0.024	6.676	6.494	2.377	15.546	0.0027	0.153	0.079
	Fusarium verticilioides	0.864	55.000	0.035	4.435	14.286	3.473	22.194	0.0055	0.193	0.099
	Penicillium notatum	1.818	55.000	0.073	9.337	14.286	7.313	30.935	0.0106	0.234	0.120
	Bipolaris maydis	1.000	5.000	0.004	5.135	1.299	0.366	6.800	0.0005	0.086	0.044
PDS	*Aspergillus flavus*	4.800	75.000	0.258	19.948	18.072	25.806	63.827	0.0453	0.329	0.150
	Aspergillus niger	4.067	75.000	0.219	16.901	18.072	21.864	56.837	0.0359	0.315	0.143
	Rhizopus stolonifer	2.300	25.000	0.041	9.559	6.024	4.122	19.705	0.0043	0.179	0.081
	Penicillium expensum	0.917	60.000	0.039	3.810	14.458	3.943	22.210	0.0055	0.193	0.088
	Fusarium verticilioides	1.654	65.000	0.077	6.873	15.663	7.706	30.242	0.0102	0.231	0.105
	Penicillium notatum	0.500	5.000	0.002	2.078	1.205	0.179	3.462	0.0001	0.051	0.023
	Rhizoctonia solani	2.000	5.000	0.007	8.312	1.205	0.717	10.233	0.0012	0.115	0.052
	Bipolaris maydis	3.000	5.000	0.011	12.468	1.205	1.075	14.748	0.0024	0.148	0.067
DS	*Aspergillus flavus*	4.925	100.000	0.348	23.661	25.000	34.806	83.467	0.0774	0.356	0.183
	Aspergillus niger	5.267	75.000	0.279	25.303	18.750	27.915	71.968	0.0575	0.342	0.176
	Rhizopus stolonifer	4.300	75.000	0.228	20.658	18.750	22.792	62.200	0.0430	0.326	0.168
	Penicillium expensum	1.900	25.000	0.034	9.128	6.250	3.357	18.735	0.0039	0.173	0.089
	Fusarium verticilioides	1.000	55.000	0.039	4.804	13.750	3.887	22.441	0.0056	0.194	0.100
	Penicillium notatum	1.423	65.000	0.065	6.837	16.250	6.537	29.624	0.0098	0.229	0.117
	Curvularia lunata	2.000	5.000	0.007	9.609	1.250	0.707	11.565	0.0015	0.126	0.065

Note: Ct=Categories, OS= Original Seed, PDS= Partial Discolour Seed, DS= Discolour Seed, Dn=Density, F= frequency, A= Abundance, RD=Relative Density, RF= Relative frequency, RA= Relative abundance, IVI= Importance value index, D= Simpson index of Dominance, H= Shannon- Weaver Index of Diversity, E= Evenness

Table 2. Biodiversity analysis of Seed mycoflora in maize by Blotter plate method

Ct	Species	Dn	F (In %)	Ab	Zone II RD (In %)	RF (In %)	RA (In %)	IVI (In %)	D=Pi*Pi	H=-{(pi) × ln(pi)}	E={H/ln(S)}
OS	*Aspergillus flavus*	4.700	100.000	0.350	21.211	24.691	35.009	80.912	0.0727	0.353	0.170
	Aspergillus niger	5.850	50.000	0.218	26.401	12.346	21.788	60.534	0.0407	0.323	0.155
	Rhizopus stolonifer	2.833	90.000	0.190	12.787	22.222	18.994	54.003	0.0324	0.309	0.148
	Fusarium verticilioides	1.800	75.000	0.101	8.123	18.519	10.056	36.698	0.0150	0.257	0.124
	Alternaria alternata	2.600	25.000	0.048	11.734	6.173	4.842	22.748	0.0057	0.196	0.094
	Penicillium notatum	2.250	40.000	0.067	10.154	9.877	6.704	26.735	0.0079	0.215	0.104
	Macrophomina phaseolina	1.625	20.000	0.024	7.334	4.938	2.421	14.693	0.0024	0.148	0.071
	Rhizoctonia soloni	0.500	5.000	0.002	2.256	1.235	0.186	3.677	0.0002	0.054	0.026
PDS	*Aspergillus flavus*	4.500	100.000	0.342	19.268	23.256	34.221	76.744	0.0654	0.349	0.159
	Aspergillus niger	4.100	50.000	0.156	17.555	11.628	15.589	44.772	0.0223	0.284	0.129
	Rhizopus stolonifer	2.605	95.000	0.188	11.155	22.093	18.821	52.069	0.0301	0.304	0.138
	Fusarium verticilioides	3.267	75.000	0.186	13.987	17.442	18.631	50.060	0.0278	0.299	0.136
	Alternaria alternata	1.500	10.000	0.011	6.423	2.326	1.141	9.889	0.0011	0.112	0.051
	Penicillium notatum	1.083	60.000	0.049	4.638	13.953	4.943	23.535	0.0062	0.200	0.091
	Macrophomina phaseolina	2.300	25.000	0.044	9.848	5.814	4.373	20.034	0.0045	0.181	0.082
	Curvularia lunata	2.000	10.000	0.015	8.563	2.326	1.521	12.410	0.0017	0.132	0.060
	Bipolaris maydis	2.000	5.000	0.008	8.563	1.163	0.760	10.487	0.0012	0.117	0.053
DS	*Aspergillus flavus*	5.225	100.000	0.407	26.381	23.810	40.661	90.852	0.0917	0.362	0.174
	Aspergillus niger	3.700	50.000	0.144	18.681	11.905	14.397	44.983	0.0225	0.285	0.137
	Rhizopus stolonifer	2.850	100.000	0.222	14.390	23.810	22.179	60.378	0.0405	0.323	0.155
	Fusarium verticilioides	1.714	70.000	0.093	8.655	16.667	9.339	34.661	0.0133	0.249	0.120
	Alternaria alternata	1.250	20.000	0.019	6.311	4.762	1.946	13.019	0.0019	0.136	0.065
	Penicillium notatum	2.167	45.000	0.076	10.939	10.714	7.588	29.241	0.0095	0.227	0.109
	Macrophomina phaseolina	1.400	25.000	0.027	7.069	5.952	2.724	15.745	0.0028	0.155	0.074
	Curvularia lunata	1.500	10.000	0.012	7.573	2.381	1.167	11.122	0.0014	0.122	0.059

Note: Ct=Categories, OS= Original Seed, PDS= Partial Discolour Seed, DS= Discolour Seed, Dn=Density, F= frequency, A= Abundance, RD=Relative Density , RF= Relative frequency, RA= Relative abundance, IVI= Importance value index, D= Simpson index of Dominance, H= Shannon- Weaver Index of Diversity, E= Evenness

(80.912%, 0.0727, 0.353, 0.170), PDS (76.744%, 0.0654, 0.349, 0.159) and DS (90.852%, 0.0917, 0.362, 0.174), respectively (Table-2).

Diversity of myco-flora in the study considered using the Shannon-Weiner diversity index (H) showed values range OS (0.353-0.054), PDS (0.349-0.112) and DS (0.362-0.122). The values for Simpson index of dominance ranges were OS (0.0727-0.0002), PDS (0.0654-0.0011) and DS (0.0917-0.0014). Pielou's evenness index of myco-flora in OS, PDS and DS samples showed value ranges of 0.170-0.026, 0.159-0.051 and 0.174-0.059, respectively.

This finding was in line with the works of Mudili *et al.* [26] showed the diversity of fungal species, including frequency, density, and diversity indices such as Important value index, Shannon-Wiener index (species richness) and Simpson index (diversity of species) in 150 freshly harvested maize samples from southern India. *Fusarium* was the prevailing genus in Karnataka (42%) and Andhra Pradesh (46%), followed by *Aspergillus* (32 and 33% respectively). In Tamilnadu, was observed highest *Fusarium* incidence (75%), followed by *Penicillium* (13%) and *Aspergillus* (12%). In Karnataka, *Aspergillus flavus* and *Aspergillus niger* were observed with 100% frequency while in Andhra Pradesh, in addition to these two *Aspergillus* species, *Penicillium chrysogenum* and *Fusarium graminearum* also showed 100% frequency. In Tamilnadu, *Fusarium verticillioides* and *F. proliferatum* were less frequent and highly dense with IVI values of 52.7 and 59.8 respectively. The species richness diversity index (Shannon index) showed that Andhra Pradesh and Karnataka were highly diversified, with several toxigenic moulds, whereas in Tamilnadu the diversity of fungal species was less.

Fungal infection is affected quality of grain through reduction in germination, increase in fatty acids, discolourization, mustiness and spoilage of the grain. Fungal development in grains is influenced by temperature, humidity and storage period. Several literature displays that a number of fungal genera *viz*., *Aspergillus*, *Fusarium*, *Penicillium*, *Bipolaris maydis*, *Alternaria*, *Cephalosporium*, *Macrophomina*, *Diplodia*, *Nigrospora*, *Botryosphaeria*, *Cladosporium*, *Trichoderma*, *Rhizoctonia* and *Mucor* have been reported from maize seed[2, 27, 28].

Tsedaley and Adugna[29] a total of 110 fungi isolates were recovered from three maize variety samples in six treatment combinations which is collected in three maize storage conditions, were harvested during 2013 cropping season. *Aspergillus, Fusarium* and *Penicillium* are the most prime fungal genera's attacking maize seed and decreasing seed germination. The highest frequency of *Aspregillus* spp. (40.4%) at farmer preserved seed with surface disinfected kernels on agar plate were recorded. The highest relative density of *Fusarium* spp. (51%) was only recorded on agar plate test from the farmer preserved seed without surface disinfected kernels. Without sterilized seeds preserved by farmers were recorded lowest germination percentage (62%). The *Aspergillus* spp. are the most dominant fungi followed by *Fusarium* spp. isolated. These fungi are important in producing secondary metabolites, which are carcinogenic to both humans and animals.

Elham *et al.*,[28] recorded percentage frequency and relative density the members of genus *Fusarium* spp. were predominantly isolated from maize grains as internal mycoflora at all locations (Fr. range 8.0 - 10% and R.D. 2.5 -3.5 as external mycoflora and internal mycoflora Fr .22.1 – 45% and R.D. 10.8 – 25%) . The second most prevalent genus as internal mycoflora was *Alternaria* spp. (Fr.20 -27.5% and RD. 10.25 -17.5%) as external mycoflora for internal mycoflora (Fr. 35-45% and R.D. 20%). The most predominant external mycoflora of the mold was *Aspergillus* spp. (Fr.27.5- 37.5 and R.D.15.13 – 23.8%) and for internal mycoflora relative density and frequency were slightly low (Fr. 16 – 18.4% and R.D. 12 – 15.3%). *Penicillium* sp. recorded the lowest value of external and internal mycoflora.

El-Shanshoury *et al.*,[33] deal with forty food grains including maize, wheat, rice and peanut seeds were analyzed for fungal contamination. Eight fungal genera belonged to *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, *Cladosporium*, *Trichoderma*, *Rhizopus* and *Alternaria* were isolated and identified. Total fungal loads as CFU and percentages of fungi in the analyzed samples ranged between 21.7-33.2x103 CFU/g and 1.6-36.7%, respectively. Contamination of grains with aflatoxins was in the following order; rice > peanut > wheat > maize. In the cultures of *Aspergillus*

flavus Link isolates, AFB1, AFB2 and AFG1 were detected in 78%, 71%, and 36% of the isolates.

Sreenivasa *et al.*,[30] a total of 86 maize samples were analyzed for frequency and relative density of internal mycoflora by direct plating method on PDA and MGA 2.5 agar medium. The most prevalent fungal genera occurring on maize grains were species of *Fusarium* and *Aspergillus*. The other genera included *Penicillium*, *Drechslera*, *Nigrospora*, *Curvularia*, *Alternaria*, *Chaetomium* and *Phoma*. The data revealed the high frequency of *Fusarium* species (96.5%) and the high relative density of *Aspergillus* species (41.7%) among the 17 fungal genera recoded. The predominant fungi recorded *Fusarium verticillioides*, *F. anthophilum*, *F. proliferatum*, *Aspergillus flavus*, *A. niger* and *A. ochraceous*, respectively.

Mostafa and Kazem,[31] reported that means of incidences *Fusarium* spp. were the highest (35.2%) followed by species *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Alternaria i.e.*, in per cent 2.9, 1.1, 2.3, 1.4 and 0.2 in that order. Among *Fusarium* species, *F. proliferatum* (90.1, 42.6%) had the highest percentages of frequency and the highest incidence in Gorgan. *Aspergillus flavus* had revealed frequency (2%) and incidence (40.2 %) and the highest level of infection was belonged to Bandare gaz seeds studied. *Penicillium* spp. were isolated from most samples examined which the highest incidence (2%) was in seeds studied in Kalale.

Niaz and Dawar[4] was used blotter, agar plate and deep freezing methods as recommended by ISTA. In all sample, 70% of the samples were infested with *Aspergillus flavus*, *A. niger*, *A. wentii* and *Penicillium* spp. Among the three methods used, agar plate method yielded the highest number of fungi as compared to blotter and deep freezing methods. Deep freezing method was the best for the detection of *Drechslera* spp., *Fusarium* spp., and *Penicillium* spp., whereas agar plate method was suitable for the detection of *Aspergillus* spp., *Cladosporium* spp., *Curvularia* spp., and *Rhizopus* spp.

Ghiasian *et al.*[32] showed a predominance of *Fusarium* species (38.5%), followed by *Aspergillus* species (8.7%), *Rhizopus* species (4.8%), *Penicillium* species (4.5%), *Mucor* species (1.1%), and four other fungal genera. *Fusarium verticillioides* was the most prevalent species. *Aspergillus flavus* was the most widely recovered *Aspergillus* species and 38% of samples were contaminated with this potentially aflatoxigenic fungus.

On the basis of present study *Aspergilus flavus* and *Aspergillus niger* were recorded dominant mycoflora. So, the next step is monitoring the mycotoxin production of isolated species.

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