International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 8, Issue 56, 2016, pp.-3035-3038.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

BIOMONITORING OF SEED MYCOFLORA CONTAMINATION OF FRESHLY HARVESTED IN MAIZE GROWING ZONE-I

KUMAR SHRVAN*, AND SINHA ASHA

Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, 221005, Uttar Pradesh, India
*Corresponding Author: Email-shrvank@gmail.com

Received: September 22, 2016; Revised: November 03, 2016; Accepted: November 04, 2016; Published: November 18, 2016

Abstract- Maize is of the main cereal produced. In this study the seed mycoflora of freshly harvested maize of zone-I were isolated by standard technique i.e. Agar plate method (APM) and Blotter plate method (BPM). A total of 9 genera i.e. *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer, Penicillium notatum, P. expensum, Trichoderma* sp. *Fusarium verticilioides, Rhizoctonia solani,* and *Macrophomina phaseolina* were isolated by standard Agar plate method and 7 fungal genera, i.e. *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer, Penicillium notatum, Fusarium verticilioides, Rhizoctonia solani* and *Macrophomina phaseolina* by blotter plate method. On the basis of density, frequency and abundance, *Aspergillus flavus* and *Aspergillus niger* 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 (88.961%, 0.0779, 0.360, 0.224), PDS (88.912%, 0.0878, 0.360, 0.224) and DS (90.536%, 0.0911, 0.362, 0.225) were contributed. In Blotter plate method, highest density of *A. niger* OS (3.946), *A. flavus* PDS (4.286), DS (4.300) were recorded. Maximum frequency showed by *A. flavus* OS (96.667 %), DS (100.000%) and *A. niger* PDS (100.000%). The abundance of *A. niger* and *A. flavus* OS (0.295), *A. flavus* PDS (0.302), DS (0.328) were recorded. Relative density maximum recorded in *A. niger* OS (22.940%), *A. flavus* PDS (24.933%) and DS (23.305%). Relative frequency highest were found in *A. flavus* OS (23.387%), DS (23.810%) and *A. niger* PDS (22.727%) and relative abundance were intended in *A. flavus* OS (29.467%), PDS (30.189 %) and DS (32.824%). Maximum IVI, Simpson index of dominance, Shannon-Weaver index of diversity and evenness contributed *A. flavus* OS (75.179%, 0.0628, 0.347, 0.215), PDS (74.628 %, 0.0619, 0.346, 0.215) and DS (79.939 %, 0.0711, 0.352, 0.219). These species are some of the common on the maize during storage and spoil the grains. So, the next step is monitoring the mycotoxin production of isolated species.

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

Citation: Kumar Shrvan and Sinha Asha, (2016) Biomonitoring of Seed Mycoflora Contamination of Freshly Harvested In Maize Growing Zone-I. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 8, Issue 56, pp.-3035-3038.

Academic Editor / Reviewer: K.D. Bhutia

Introduction

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 fungi that cause ear and kernel rots including, *Aspergillus*, *Fusarium verticillioides*, *F. proliferatum, F. subglutinans*, *Gibberellazeae 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 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-Wiene 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 determining the biomonitoring of seed mycoflora contamination of freshly harvested in maize growing Zone-I.

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, six maize seed samples were taken from maize growing Zone I. 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 (9 cm 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. Associated fungi, which cannot be identified, 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].

Quantitative analysis

Based on the individuals fungi recorded in the distinct seed samples were quantitatively 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:

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

Frequency (%) is calculated by the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occured X 100}}{\text{Total number of Petry plate studied}}$$

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:

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

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

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

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

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

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 i^{th} 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 i^{th} 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-I, (Almora, Kullu, Bilaspur, Daulakauna Kangra and Saharanpur). In this study, six maize seed samples were taken from maize growing Zone Icotegory.

A total of 9 genera were recorded within three seed categories through Agar plate method. Association of *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer, Penicillium notatum, P. expensum, Trichoderma* sp. *Fusarium verticilioides, Rhizoctonia solani* and *Macrophomina phaseolina* were observed [Table-1]. Maize mycoflora was presented with 7 fungal genera, i.e. *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer, Penicillium notatum, Fusarium verticilioides, Rhizoctonia solani* and *Macrophomina phaseolina* by Blotter plate method [Table-2].

In APM, Highest density, frequency, abundance of *A. flavus* OS (4.717, 100.00, 0.421), PDS (4.783, 100.00, 0.410) and DS (4.783, 100.00, 0.437) were recorded. Frequency (100 %) was also found in OS, PDS, and DS. Highest relative density, frequency, abundance by *A. flavus* OS (22.717, 24.194, 42.051), PDS (22.702, 25.210, 41.000) and DS (20.067, 26.786, 43.683) 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 (88.961%, 0.0779, 0.360, 0.224), PDS (88.912%, 0.0878, 0.360, 0.224) and DS (90.536%, 0.0911, 0.362, 0.225) were contributed.

Diversity of myco-flora in the study calculated using the Shannon-Weiner diversity index (H') showed values range OS (0.360-0.061), PDS (0.360-0.098) and DS (0.362-0.128). The values for Simpson index of dominance ranges were OS (0.0779-0.0002), PDS (0.0878-0.0007) and DS (0.0911-0.0016). Pielou's evenness index of myco-flora in OS, PDS and DS samples showed value ranges of 0.224-0.038, 0.224-0.061 and 0.225-0.079, respectively [Table-1].

In BPM, Highest density were recorded *A. niger* OS (3.946), *A. flavus* PDS (4.286), DS (4.300). Maximum frequency showed values *A. flavus* OS (96.667 %), DS (100.000%) and *A. niger* PDS (100.000%) and abundance were calculated *A. niger* and *A. flavus* OS (0.295), *A. flavus* PDS (0.302), DS (0.328). Relative density maximum recorded in *A. niger* OS (22.940%), *A. flavus* PDS (24.933%) and DS (23.305%). Relative frequency highest were found in *A. flavus* OS (23.387%), DS (23.810%) and *A. niger* PDS (22.727%) and relative abundance were intended in *A. flavus* OS (29.467%), PDS (30.189 %) and DS (32.824%). Maximum IVI, Simpson index of dominance, Shannon-Weaver index of diversity and evenness contributed *A. flavus* OS (75.179%, 0.0628, 0.347, 0.215), PDS (74.628 %, 0.0619, 0.346, 0.215) and DS (79.939 %, 0.0711, 0.352, 0.219), respectively [Table-2].

Diversity of myco-flora in the study considered using the Shannon-Weiner diversity index (H) showed values range OS (0.347-0.100), PDS (0.346-0.123) and DS (0.352-0.132). The values for Simpson index of dominance ranges were OS (0.0628-0.0027),PDS (0.0619-0.0014) and DS (0.0710-0.0017). Pielou's evenness index of myco-flora in OS, PDS and DS samples showed value ranges of 0.215-0.062, 0.215-0.077 and 0.219-0.082, 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.

Table-1 *Quantitative analysis of Seed mycoflora in maize by APM*

Zone I APM											
Ct	Species	Dn	F (in %)	Ab	RD (in %)	RF (in %)	RA (in %)	IVI (RD+RF+RA)	D=ni*ni	H=-{(ni) × ln(ni)}	E={H/ln(S)}
OS	*Aspergillus flavus*	4.717	100.000	0.421	22.717	24.194	42.051	88.961	0.0879	0.360	0.224
	A. niger	1.567	50.000	0.070	7.546	12.097	6.984	26.626	0.0079	0.215	0.134
	Rhizopus stolonifer	2.192	86.667	0.169	10.559	20.968	16.939	48.466	0.0261	0.294	0.183
	Penicillium notatum	2.292	40.000	0.082	11.037	9.677	8.172	28.887	0.0093	0.225	0.140
	Trichoderma sp.	4.417	20.000	0.079	21.272	4.839	7.875	33.986	0.0128	0.247	0.153
	Fusarium verticilioides	2.206	56.667	0.111	10.624	13.710	11.144	35.478	0.0140	0.252	0.157
	Rhizoctonia solani	1.273	36.667	0.042	6.130	8.871	4.160	19.161	0.0041	0.176	0.109
	Macrophomina phaseolina	0.500	6.667	0.003	2.408	1.613	0.297	4.318	0.0002	0.061	0.038
	Penicillium expensum	1.600	16.667	0.024	7.706	4.032	2.377	14.116	0.0022	0.144	0.089
PDS	*Aspergillus flavus*	4.783	100.000	0.410	22.702	25.210	41.000	88.912	0.0878	0.360	0.224
	A. niger	1.885	43.333	0.070	8.945	10.924	7.000	26.869	0.0080	0.216	0.134
	Rhizopus stolonifer	2.979	80.000	0.204	14.140	20.168	20.429	54.736	0.0333	0.310	0.193
	Penicillium notatum	1.958	40.000	0.067	9.295	10.084	6.714	26.093	0.0076	0.212	0.132
	Fusarium verticilioides	2.548	70.000	0.153	12.091	17.647	15.286	45.024	0.0225	0.285	0.177
	Rhizoctonia solani	0.917	20.000	0.016	4.351	5.042	1.571	10.964	0.0013	0.121	0.075
	Macrophomina phaseolina	1.000	10.000	0.009	4.746	2.521	0.857	8.124	0.0007	0.098	0.061
	Trichoderma sp.	3.500	16.667	0.050	16.612	4.202	5.000	25.813	0.0074	0.211	0.131
	Penicillium expensum	1.500	16.667	0.021	7.119	4.202	2.143	13.464	0.0020	0.139	0.087
DS	*Aspergillus flavus*	4.783	100.000	0.437	20.067	26.786	43.683	90.536	0.0911	0.362	0.225
	A. niger	1.958	40.000	0.072	8.216	10.714	7.154	26.084	0.0076	0.212	0.132
	Rhizopus stolonifer	1.580	83.333	0.120	6.628	22.321	12.024	40.974	0.0187	0.272	0.169
	Penicillium notatum	2.846	43.333	0.113	11.940	11.607	11.263	34.811	0.0135	0.250	0.155
	Trichoderma sp.	2.250	6.667	0.014	9.439	1.786	1.370	12.595	0.0018	0.133	0.083
	Fusarium verticilioides	3.036	46.667	0.129	12.735	12.500	12.938	38.173	0.0162	0.262	0.163
	Rhizoctonia solani	1.083	20.000	0.020	4.545	5.357	1.979	11.881	0.0016	0.128	0.079
	Trichoderma sp.	4.400	16.667	0.067	18.459	4.464	6.697	29.620	0.0097	0.229	0.142
	Penicillium expensum	1.900	16.667	0.029	7.971	4.464	2.892	15.327	0.0026	0.152	0.094
	Note: BPM=Blotter Plate Method, 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 *Quantitative analysis of Seed mycoflora in maize by BPM*

Zone I BPM											
Ct	Species	Dn	F (in %)	Ab	RD (in %)	RF (in %)	RA (in %)	IVI (RD+RF+RA)	D=ni*ni	H=-{(ni) × ln(ni)}	E={H/ln(S)}
OS	*Aspergillus flavus*	3.810	96.667	0.295	22.325	23.387	29.467	75.179	0.0628	0.347	0.215
	Aspergillus niger	3.946	93.333	0.295	22.940	22.764	29.467	75.171	0.0628	0.347	0.215
	Rhizopus stolonifer	2.957	76.667	0.181	17.323	18.548	18.133	54.004	0.0324	0.309	0.192
	Rhizoctonia soloni	1.955	36.667	0.057	11.452	8.871	5.733	26.056	0.0075	0.212	0.132
	Macrophomina phaseolina	0.750	13.333	0.008	4.394	3.226	0.800	8.420	0.0008	0.100	0.062
	Penicillium notatum	2.500	70.000	0.140	14.648	16.935	14.000	45.583	0.0231	0.286	0.178
	Fusarium verticilioides	1.286	23.333	0.024	7.533	5.645	2.400	15.578	0.0027	0.154	0.095
PDS	*Aspergillus flavus*	4.286	93.333	0.302	24.933	21.212	30.189	76.334	0.0647	0.348	0.216
	Aspergillus niger	3.883	100.000	0.293	22.592	22.727	29.308	74.628	0.0619	0.346	0.215
	Rhizopus stolonifer	2.920	83.333	0.184	16.988	18.939	18.365	54.292	0.0328	0.309	0.192
	Rhizoctonia soloni	1.500	33.333	0.038	8.727	7.576	3.774	20.076	0.0045	0.181	0.112
	Macrophomina phaseolina	0.786	23.333	0.014	4.571	5.303	1.384	11.258	0.0014	0.123	0.077
	Penicillium notatum	2.370	76.667	0.137	13.786	17.424	13.711	44.920	0.0224	0.284	0.177
	Fusarium verticilioides	1.444	30.000	0.033	8.403	6.818	3.270	18.492	0.0038	0.172	0.107
DS	*Aspergillus flavus*	4.300	100.000	0.328	23.305	23.810	32.824	79.939	0.0710	0.352	0.219
	Aspergillus niger	3.100	83.333	0.197	16.801	19.841	19.720	56.363	0.0353	0.314	0.195
	Rhizopus stolonifer	3.250	73.333	0.182	17.614	17.460	18.193	53.268	0.0315	0.307	0.191
	Rhizoctonia soloni	1.594	53.333	0.065	8.638	12.698	6.489	27.825	0.0086	0.221	0.137
	Macrophomina phaseolina	0.813	26.667	0.017	4.404	6.349	1.654	12.407	0.0017	0.132	0.082
	Penicillium notatum	3.895	63.333	0.188	21.109	15.079	18.830	55.017	0.0336	0.311	0.193
	Fusarium verticilioides	1.500	20.000	0.023	8.130	4.762	2.290	15.182	0.0026	0.151	0.094
Note: BPM=Blotter Plate Method, 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											

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*, *Bipolarismaydis, 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 percentages (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– 8.4% and R.D. 12–15.3%). *Penicillium* sp. recorded the lowest value of external and internal mycoflora.

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 *F. verticillioides*, *F. anthophilum*, *F. proliferatum*, *A. 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 Bandaregaz 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.

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

Conflict of Interest: None declared

References

[1] FAO/CIMMYT (1997) *White maize: a traditional food grain in developing countries*. pp.1-27.

[2] Payne G.A. (1999) Ear and Kernel Rots. In Donald G. White (ed), Compendium of Corn Diseases. St. Paul, Minnesota: *The American Phytopathology Society*. pp. 44-47.

[3] http://cropprotectionnetwork.org/corn/ear-rots/

[4] Niaz I. and S. Dawar (2009) *Pak. J. Bot.,* 41(1), 443- 45.

[5] Bhattacharya K. and Raha S. (2002) *Mycopathologia,* 155, 135. doi:10.1023/A:1020475411125

[6] Gonzalez H.H.L., Resnik S.L., Boca R.T. and Marasas W.F.O. (1995) *Mycopathologia,* 130, 29.

[7] Kumar Shrvan, Sinha Asha, Hooda K. S. and Singh Vimla (2015a) *Advanced detection techniques of toxigenic fungi and their management strategies.* In: Sixth IJAA-JSPS, International conference on Contemporary Advances of Science and Technology, 7-9 Aug., BHU, Varanasi p.145.

[8] Kumar Shrvan, Dawa Dolma Bhutia, Asha Sinha, L. Dikho Chajiio and Sonai Kundu (2016) *FPB 34-Biosensor: A vigilant device for Myco-toxins in Food processing industry.* In: Recent Advances in food Processing and biotechnology, 5-6 April, BHU, Varanasi p.159

[9] Sobuj N.A. and Rahman M. (2011) *Inter. J. Environ. Sci.,* 2(1), 1-13.

[10] Deka J., Tripathi P.O. and Khan L.M. (2012) *Int. J. Ecosys*., 2(4), 67-73.

[11] Muskett A.E. (1948) *Trans. Br. Mycol. Soc.,* 30, 74-83.

[12] De Tempe J. (1953) *Proc. Int. Seed Test. Association,* 21, 133-151.

[13] Thom C. and Raper K.B. (1945) *A manual of the Aspergilli.* The Williams & Wilkins Company, Baltimore. DOI: http://dx.doi.org/10.5962/bhl.title.5694

[14] Raper K.B. and Thom C. (1949) *A manual of the penicillia.* The Williams & Wilkins Company, Baltimore. pp.1–875, DOI:http://dx.doi.org/10.5962/bhl.title.4993

[15] Barnett H.L. (1962) Illustrated genera of imperfect fungi. Burgess Publishing Company, USA.

[16] Klich M.A. (2002) Identification of common *Aspergillus* species. Centraalbureau voor Schimmel cultures, Utrecht.

[17] Leslie John F. and Summerell Brett A. (2008) The *Fusarium* Laboratory Manual. John Wiley & Sons.pp. 388.

[18] Visagie C.M., Houbraken J., Frisvad J.C., Hong S.B., Klaassen C.H.W., Perrone G., Seifert K.A., Varga J., Yaguchi T., and Samson R.A. (2014) *Studies in Mycology,* 78, 343–371. doi: 10.1016/j.simyco.2014.09.001.

[19] URL 1: www.mycobank.org

[20] URL 2: www.indexfungorum.org

[21] Curtis J.T., McIntosh R.P. (1950) *Ecology,* 31,434-455.

[22] Burlakoti C. and Karmacharya S.B. (2004) *Nepal Him. J. Sci.,* 2(3), 37-41

[23] Simpson E.H. (1949) *Nature,* 163, 688.

[24] Shannon C.E. and Weaver W. (1963) *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, Illinois.

[25] Pielou E.C. (1966) *An introduction to mathematical Ecology*. John Wiley and Sons, New York

[26] Mudili, Venkataramana, Siddaih Chandra Nayaka, Madhukar Nagesh, Garapati Phanikumar, Kalagatur Naveen Kumar, Harish chandra Sreepathi Murali, Tapani YliMattilad and Harsh Vardan Batra (2014) *J Sci Food Agric*, 94, 2674–2683. DOI 10.1002/jsfa. 6608

[27] Tulin J.M. and Askun D.I. (2006) *J. Biol. Sci.,* 6(2), 275–28.

[28] Elham S., Dawood, Modhi K., Elshamry (2015) *International Journal of Scientific & Technology Research,* 4(06), 227–230.

[29] Tsedaley B. and Adugna G. (2016) *J. Plant Pathol. Microbiol.,* 7(3), 38–44. doi:10.4172/2157-7471.1000338

[30] Sreenivasa M.Y. Dass R.S. Raj A.P.C., Janardhana G.R. (2011) *World Applied Sciences Journal,* 13(4), 688–692.

[31] Mostafa, Abedi-Tizaki and Kazem, Sabbagh Seyed (2011) *Annals of Biological Research*, 2 (5),681–688.