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Myco-Diversity of Freshly Harvested Seed in Maize Growing Zone-III

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Abstract Maize is known as queen of cereals crops because reflected third most important cereal crops in the world. In our study the seed mycoflora of freshly harvested maize of zone-III were isolated by Agar plate method (APM) and Blotter plate method (BPM). A total of 5 genera i.e. *Aspergillus flavus, A. niger, Fusarium verticilioides, P. expensum* and *Rhizopus stolonifer*, were isolated by standard Agar plate method and 9 fungal genera, i.e. *Aspergillus flavus, A. niger, A. versicilar, Fusarium verticilioides, Macrophomina phaseolina, Penicillium notatum, Rhizopus stolonifer, Rhizoctonia solani* and *Trichoderma* sp. 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 discolor (PDS) and Discolor seed (DS). Highest Important value index (IVI), Simpson index of dominance (D), Shannon-Weaver index of diversity (H) and evenness (E) of *A. niger* OS (104.740%, 0.1219, 0.367, 0.228), PDS (99.906%, 0.1109, 0.366, 0.228) and *R. stolonifer* DS (83.866%, 0.0781, 0.356, 0.221) were contributed. In Blotter plate mathod, highest density, frequency and abundance were recorded *A. flavus* OS (6.650, 100.000, 0.489), PDS (6.950, 100.000, 0.523), DS (6.700, 100.000, 0.496). Per cent maximum relative density, frequency and abundance values *A. flavus* OS (39.583, 20.408, 48.897), PDS (41.431, 22.222, 52.256) and DS (44.395, 22.727, 49.630) were intended. Mycodiversity showed Shannon-Weiner diversity index (H) values range OS (0.368–0.072), PDS (0.367–0.098) and DS (0.367–0.078). The values for Simpson index of dominance ranges were OS (0.1317–0.0003), PDS (0.1493–0.0007) and DS (0.1515–0.0004). Pielous evenness index of myco-flora in OS, PDS and DS samples showed value ranges of 0.229–0.045, 0.228–0.061 and 0.228–0.048, respectively. 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, Shannon-Weaver index of diversity.

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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 fungal species 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]. 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]. Fungal species are related to corn mostly belong to *Aspergillus* 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 [5, 6]. 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 showeda rich ecosystem with high species diversity and low value (H) will have a low species diversity [7]. The present study was aimed at myco-diversity measurement of seed mycoflora contamination of freshly harvested in maize growing zone-III.

Materials 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, two maize seed samples were taken from maize growing zone-III. 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 discolored seed (PDS) and discolored seed (DS). Myco-flora detected on maize seed by Agar plate method-APM [8] and Blotter plate method-BPM [9]. 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-I-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 replication 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 [10—12].

Based on the individuals fungi recorded in the distinctseed samples were analyzed 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 [13].

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:

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

Table 1. Quantitative analysis of seed mycoflora in maize by agar plate method. Ct=Categories, OS=Original seed, PDS=Partial discolor seed, DS=Discolor 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.

		Zone III									
Ct	Species	Dn	F (In %)	Ab	RD (In %)	RF (In %)	RA (In %)	IVI (In %)	D= Pi*Pi	H={(pi) ×In(pi)}	E={H/ In (S)}
OS	*Aspergillus flavus*	2.167	90.000	0.160	16.376	21.951	15.984	54.311	0.0328	0.309	0.192
	A. niger	5.100	100.000	0.418	38.546	24.390	41.803	104.740	0.1219	0.367	0.228
	Rhizopus stolonifer	3.650	100.000	0.299	27.587	24.390	29.918	81.895	0.0745	0.354	0.220
	Penicillium expensum	1.714	70.000	0.098	12.957	17.073	9.836	39.866	0.0177	0.268	0.167
	Fusarium verticilioides	0.600	50.000	0.025	4.535	12.195	2.459	19.189	0.0041	0.176	0.109
PDS	*Aspergillus flavus*	5.350	100.000	0.149	47.299	25.641	14.894	87.833	0.0857	0.360	0.223
	A. niger	3.250	100.000	0.455	28.733	25.641	45.532	99.906	0.1109	0.366	0.228
	Rhizopus stolonifer	1.111	90.000	0.277	9.823	23.077	27.660	60.560	0.0407	0.323	0.201
	Penicillium expensum	0.800	50.000	0.085	7.073	12.821	8.511	28.404	0.0090	0.223	0.139
	Fusarium verticilioides	0.800	50.000	0.034	7.073	12.821	3.404	23.297	0.0060	0.198	0.123
DS	*Aspergillus flavus*	3.000	100.000	0.261	17.682	24.390	20.690	62.762	0.0438	0.327	0.203
	A. niger	3.350	100.000	0.291	19.745	24.390	23.103	67.238	0.0502	0.335	0.208
	Rhizopus stolonifer	4.650	100.000	0.404	27.407	24.390	32.069	83.866	0.0781	0.356	0.221
	Penicillium expensum	5.167	60.000	0.270	30.452	14.634	21.379	66.465	0.0491	0.334	0.207
	Fusarium verticilioides	0.800	50.000	0.035	4.715	12.195	2.759	19.669	0.0043	0.179	0.111

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

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

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

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

Importance Value Index (IVI)-It was calculated by equation [14]-

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 [15]:

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

Where ni is the proportion of individuals of the i^{th} species in the community. Simpson's index given relatively little weight to the rare species and more weight to the common species. It weight 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

Table 2. Quantitative analysis of seed mycoflora in maize by blotter plate method. Ct=Categories, OS=Original seed, PDS=Partial discolor seed, DS=Discolor seed, Dn=Density, F=Frequency, A=Abundance, RD=Relatve density, RF=Relative frequency, RA=Relative abundance, IVI=Importance value index, D=Simpson index of dominance, H=Shannon-Weaver index of diversity, E=Evenness.

					Zone III						
Ct	Species	Dn	F (In %)	Ab	RD (In %)	RF (In %)	RA (In %)	IVI (In %)	D= Pi*Pi	H={(pi) ×In(pi)}	E={H/ In (S)}
OS	*Aspergillus flavus*	6.650	100.000	0.489	39.583	20.408	48.897	108.889	0.1317	0.368	0.229
	A. niger	3.150	100.000	0.232	18.750	20.408	23.162	62.320	0.0432	0.326	0.203
	Rhizopus stolonifer	2.250	100.000	0.165	13.393	20.408	16.544	50.345	0.0282	0.300	0.186
	Rhizoctonia solani	1.500	20.000	0.022	8.929	4.082	2.206	15.216	0.0026	0.151	0.094
	A. versicolor	0.667	30.000	0.015	3.968	6.122	1.471	11.561	0.0015	0.125	0.078
	Penicillium notatum	0.750	60.000	0.033	4.464	12.245	3.309	20.018	0.0045	0.181	0.112
	Trichoderma sp.	0.500	10.000	0.004	2.976	2.041	0.368	5.385	0.0003	0.072	0.045
	Macrophomina phaseolina	0.500	10.000	0.004	2.976	2.041	0.368	5.385	0.0003	0.072	0.045
	Fusarium verticilloides	0.833	60.000	0.037	4.960	12.245	3.676	20.882	0.0048	0.185	0.115
PDS	*Aspergillus flavus*	6.950	100.000	0.523	41.431	22.222	52.256	115.909	0.1493	0.367	0.228
	A. niger	1.900	100.000	0.143	11.326	22.222	14.286	47.834	0.0254	0.293	0.182
	Rhizopus stolonifer	3.050	100.000	0.229	18.182	22.222	22.932	63.336	0.0446	0.328	0.204
	Rhizoctonia solani	0.875	40.000	0.026	5.216	8.889	2.632	16.737	0.0031	0.161	0.100
	A. versicolor	1.500	10.000	0.011	8.942	2.222	1.128	12.292	0.0017	0.131	0.081
	Penicillium notatum	0.500	20.000	0.008	2.981	4.444	0.752	8.177	0.0007	0.098	0.061
	Trichoderma sp.	1.500	40.000	0.045	8.942	8.889	4.511	22.342	0.0055	0.193	0.120
	Fusarium verticilioides	0.500	40.000	0.015	2.981	8.889	1.504	13.373	0.0020	0.139	0.086
DS	*Aspergillus flavus*	6.700	100.000	0.496	44.395	22.727	49.630	116.752	0.1515	0.367	0.228
	A. niger	2.350	100.000	0.174	15.572	22.727	17.407	55.706	0.0345	0.313	0.194
	Rhizopus stolonifer	3.500	100.000	0.259	23.192	22.727	25.926	71.845	0.0574	0.342	0.213
	A. versicolor	0.500	30.000	0.011	3.313	6.818	1.111	11.242	0.0014	0.123	0.076
	Penicillium notatum	0.500	10.000	0.400	3.313	2.273	0.370	5.956	0.0004	0.078	0.048
	Trichoderma sp.	0.875	40.000	0.026	5.798	9.091	2.593	17.481	0.0034	0.166	0.103
	Fusarium verticilioides	0.667	60.000	0.030	4.417	13.636	2.963	21.017	0.0049	0.186	0.116

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 [16]:

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

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 [17] as:

$$E = H^1/\log S$$

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-III (Varanasi and Begusarai). In this study, two maize seed samples were taken from maize growing Zone III category. A total of 5 genera were recorded within three seed categories through Agar plate method. Association of *Aspergillus flavus, A. niger, Fusarium vericillioides, P. expensum* and *Rhizopus stolonifer*, were observed (Table 1). Maize mycoflora was presented with 9 fungal genera, i.e. *Aspergillus flavus, A. niger, A. versicilar, Fusarium verticillioides, Macrophomina phaseolina, Penicillium notatum, Rhizopus stolonifer, Rhizoctonia solani* and *Trichoderma* sp. by Blotter plate method (Table 2). In Agar plate method, highest density, frequency and abundance of *A. niger* OS (5.100, 100.000, 0.418), *A. flavus* PDS (Dn-5.350, F-100.000), *A. niger* (Ab-0.455) and *P. expensum* DS (Dn-5.167), *R. stolonifer* (F-100.000, Ab-0.404) were recorded.

Highest relative density, frequency, abundance by *A. flavus* OS (38.546, 24.390, 41.803), *A. flavus* PDS (RD-47.299, RF-25.641), *A. niger* (RA-45.532) and *P. expensum* DS (RD-30.452), *R. stolonifer* (RF-24.390, RA-32.069) were observed. Highest important value index (IVI). Simpson index of dominance (D), Shannon-Weaver index of diversity (H) and evenness (E) of *A. niger* OS (104.740%, 0.1219, 0.367, 0.228), PDS (99.906%, 0.1109, 0.366, 0.228) and *R. stolonifer* DS (83.866%, 0.0784, 0.356, 0.221) were contributed.

Diversity of myco-flora in the study calculated using the Shannon-Weiner diversity index (H) showed values range OS (0.367–0.176), PDS (0.360–0.198) and DS (0.356–0.179). The values for Simpson index of dominance ranges were OS (0.1219–0.0041), PDS (0.1109–0.0060) and DS (0.0781–0.0043). Pielou's evenness index of myco-flora in OS, PDS and DS samples showed value ranges of 0.228–0.109, 0.228–0.123 and 0.221–0.111, respectively (Table 1).

In Blotter plate mathod, highest density, frequency and abundance were recorded *A. flavus* OS (6.650, 100.000, 0.489), PDS (6.950, 100.000, 0.523), DS (6.700, 100.000, 0.496). Percent maximum relative density, frequency and abundance values *A. flavus* OS (39.583, 20.408, 48.897), PDS (41.431, 22.222, 52.256) and DS (44.395, 22.727, 49.630) were intended. Maximum IVI, Simpson index of dominance, Shannon-Weaver index of diversity and evenness contributed *A. flavus* OS (108.889%, 0.1317, 0.368, 0.229), PDS (115.909%, 0.1493, 0.367, 0.228) and DS (116.752%, 0.1515, 0.367, 0.228), respectively (Table 2).

Diversity of myco-flora in the study considered using the Shannon-Weiner diversity index (H) showed values range OS (0.368–0.072), PDS (0.367–0.098) and DS (0.367–0.078). The values for Simpson index of dominance ranges were OS (0.1317–0.0003), PDS (0.1493–0.0007) and DS (0.1515–0.0004). Pielou's evenness index of myco-flora in OS, PDS and DS samples showed value ranges of 0.229–0.045, 0.228–0.061 and 0.228–0.048, respectively.

This finding was in line with the works of Mudili et al. [18] 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.

Tsedaley and Adugna [19] recovered a total of 110 fungi isolates 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 generas attacking maize seed and decreasing seed germination. The high-

est 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.

El-Shanshoury et al. [20] 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 21.7–33.2 × 103 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. [21] analyzed a total of 86 maize samples for frequency and relative density of internal mycoflora by direct planting 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 recorded. The predominant fungi recorded *Fusarium verticillioides, F. anthophilum, F. proliferatum, Aspergillus flavus, A. niger* and *A. ochraceous*, respectively.

Mostafa and Kazem [22] 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 [3] 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 *Drec-hslera* 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 *Aspergilus flavus, Aspergillus niger* and *R. stolonifer* were recorded dominant mycoflora. So, the next step is monitoring the mycotoxin production of isolated species.

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