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Diversity Analysis of Mycoflora Associated with Maize Seeds Collected from different Regions of Tamil Nadu

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ABSTRACT: Maize is one of the most important cereal crops in the world and has been titled "The Queen of cereals". The yield of the maize crop is being hampered by different biotic and abiotic factors, among which post-harvest and storage infection plays a significant role. Hence, in the present study, we focused on assessing the mycoflora associated with the seed surface, which is responsible for the post-harvest losses by using the standard seed blotter method. The results revealed that the presence of eight fungal species belonging to six fungal genera was found to be associated with the seed of maize. *Aspergillus* spp., accounts for 86.3 percent of the total mycoflora population among the six fungal genera. The other genera include *Fusarium*, *Penicillium*, *Rhizopus*, *Alternaria* and *Macrophomina*. The results of the Relative Density (RD) study revealed that *Penicillium*, *Rhizopus*, *Alternaria* and *Macrophomina* come under rare fungal species and the most abundant species were *A. niger*, *A. flavus* and *A. fumigatus*. As per Edwino Fernando's Ranking of Biodiversity Indices, the Shannon-Weiner index (H) was less than 1.9, which indicates that the diversity of mycoflora is shallow, whereas Simpson's index was more than 0.56 in all locations except in Perambalur. The evenness value was more than 0.5 in all the sites, indicating that the species distribution is even. Beta-diversity was measured (paired comparison) and there was no similarity between sites.

Keywords: *Aspergillus*, diversity, *Fusarium*, maize, mycoflora, seeds.

INTRODUCTION

Maize seeds harbormany ectophytic and endophytic microbiomes including fungi, bacteria and actinomycetes. Several ectophytic mycoflora isolated were *Aspergillus*, *Fusarium verticillioides*, *F. proliferatum, F. glutinans*, *Gibberella zeae, Penicillium*, *Macrophomina phaseolina, Diplodia, Nigrospora, Botryosphaeria, Cladosporium, Trichoderma, Rhizoctonia,* and *Rhizopus* (Bhatnagar *et al.,* 1999). Mycoflora infection in maize seeds causesa reduction in germination various abnormalities and leads to rejection (Singh *et al*., 2021). Storage fungus infects the seeds as they are moved into storage and, in the right circumstances, can quickly spread throughout the bulk. These fungi develop on maize seeds; they become visible, can kill the seed, generate an unpleasant odor or taste, and occasionally the seeds are unfit for human eating because the seed fungi release mycotoxin along with a change in the chemical makeup of the seed (Ingle *et al.,* 2021). Mycoflora associated with maize seed are members of *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., and these are mycotoxin producing fungi. *Aspergillus* spp., produces various mycotoxins and aflatoxin B1 is extremelytoxic andis classified as a group Ia human carcinogenic by the International Agency for Research on Cancer, in addition to considerable economic losses in the food and agricultural sectors. A study on fungal species diversity is one of the most important indices used to evaluate an ecosystem. Several diversity indices such as population dynamics, species richness, evenness, dominance of mycoflora, etc. are used. Usually, fungal species diversity is one of the most important indices used for the evaluation of an ecosystem. Fungal species richness, an intuitive element of fungal diversity, is commonly used to compare habitats, as species diversity is usually assumed to reflect niche diversity when limiting similarity drives species coexistence (Silvertown, 2004). Fungal diversity can change because of time, climate, biota, topography, natural disturbance, or human-caused perturbation and contamination (Day *et al*., 2019). For these reasons, there's an interest in developing approaches to predict various facets of fungal diversity and how it is likely to change over space and time in natural and managed ecosystems. Alpha diversity reveals the biodiversity component of the community and whereas beta diversity reveals how it changes across locations. Understanding the compositional pattern of species helps researchers to understand different aspects of species interaction and ecosystem function (Legendre, 2014). Beta diversity patterns provide knowledge about

the uniqueness of community composition in the landscape. Keeping this in view, the present investigation was designed to study the diversity of mycoflora of maize seeds collected from different maize growing areas of Tamil Nadu.

MATERIALS AND METHODS

Collection of seed samples. Maize seed samples were collected in ten locations covering major maize growing areas of Tamil Nadu that include Virudhunagar, Namakkal, Tiruppur, Madurai, Dharmapuri, Salem, Oddanchathiram, Ariyalur, Perambalur and Dindigul. One kg of seed samples were collected directly from maize growing farmers and seed sellers. In each location, seeds were collected in five different areas and the seeds were homogenized to represent one location. These homogenized seeds were subjected to mycoflora assessment in the Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu.

Mycoflora assessment. Mycoflora on maize seeds was assessed by the standard blotter method (De Tempe, 1963). One Hundred seeds from each location placed inplastic Petri dishes (90 mm dia.) lined with two layers of blotter papers and one layer of filter paper moistened with distilled water. Ten seeds will be placed in each Petri dish equidistantly (pattern 9-1). The seeded Petri dishes were incubated at 25 ± 1°C for seven days and the seeds were examined regularly for the presence of different fungi. Incubated seeds were examined visually under a Stereo-zoom microscope for the growth pattern of mycoflora (Kumar *et al.,* 2017).

Morphological identification of fungal genera. Individual fungal colonies which were observed under a stereo-zoom microscope were subcultured in potato dextrose agar medium. The fungal colonies were further purified by the single hyphal tip method. These pure cultures were then subjected to microscopic observation for morphological identification of the fungal species. Fungal genera were confirmed by both cultural and morphological characters.

Computation of Diversity Indices

Based on the individuals, fungi recorded in the distinct seed samples were analyzed for species richness and species distribution, evenness, alpha-diversity and beta-diversity.

Shannon-Weiner index (H') and Simpson's index were widely used to describe the α-diversity.

Computation of Relative Density

The Relative Density (RD) of fungal species and genera was calculated according to the method suggested by Tadych *et al*. (2012).

$$\text{Relative Density (RD) (\%)} = \frac{ni}{Ni}\ x\ 100$$

Where,

ni is the number of genus or species isolated,

Ni is the total number of isolates.

Computation of α-diversity. Alpha diversity can be found by calculating three parameters like Shannon – Wiener Diversity Index, Species Evenness Index and Simpson Diversity Index (D). The formulas for computing the above three parameters are as follows:

Simpson Diversity Index (D). The Simpson Diversity Index represents the species diversity in a particular location

$$\text{Simpson Diversity Index (D)} = \frac{\sum \text{n}\ (\text{n}-1)}{\text{N}(\text{N}-1)}$$

Where,

n is the total number of individuals in a particular species

N is the total number of individuals

$$\text{Shannon –Wiener Diversity Index (H')} = \sum_{i=1}^{s} p_i \ln p_i$$

Where,

*P*i (relative abundance) is equal to n_i/N

n_i is the number of individuals in i^{th} species

N is the total number of individuals (Shannon and Weaver, 1963)

$$\text{Species Evenness Index} = \frac{H'}{\ln(R)}$$

Where,

H' is the Shannon Wiener Index – ranging from 0 to 6.

R is the species richness which is also equivalent to s (the number of species found in the given area).

The next step is to proceed with ranking the values obtained for each index. In this paper, Fernando's Biodiversity Scale was used to rank the indices (Table 1).

Computation of β – Diversity. Beta diversity refers to the species diversity between any two regions. It is used for large-scale comparison of species diversity. β-diversity was calculated by using the following formula given by Fontana *et al.* (2020).

β-diversity = $(N_1\text{-C})+(N_2\text{-C})$

Where,

N_1 refers to the total number of species present in location 1

N_2 refers to the total number of species present in location 2

C refers to the number of species that both locations have in common

Statistical analysis. Various diversity indices (α-Diversity, β-Diversity) were calculated, and graphs were drawn using 'PAST' software.

RESULTS AND DISCUSSION

Mycoflora assessment. Maize seeds collected from different maize growing areas of Tamil Nadu were used to assess the fungal diversity. A total of 803 fungal isolates belonging to ten different species were observed, out of which five belong to the genus *Aspergillus,* contributing 86.3 percent of the total fungal population. The fungal population was dominated by *Aspergillus* spp., followed by *Fusarium* spp., (6.23%). Other fungal genera recorded in the present study were *Penicillium* spp., (4.23%), *Rhizopus* spp., (2.37%), *Alternaria* spp., (0.37%) and *Macrophomina* (0.50%) (Data not shown; Fig. 1). The fungal genera were identified based on the following various cultural and morphological characters.

Aspergillus flavus. The fungus produced olive or dark green colonies with profuse sporulation. Mycelium is hyaline, septate and branched. It produced circular

single-celled green colored conidia arranged in chains from the biseriate phialide arising from the conidiophore (Fig. 2a).

Aspergillus niger. The fungus had dark brown to black colonies with enormous sporulation. Mycelium is hyaline, septate and branched. Conidiophores were smooth, aseptate and unbranched. Biseriate conidial heads produced smooth black colored conidia in chains (Fig. 2b).

Aspergillus fumigates. Initially, *A. fumigatus* produced white colonies, which later turned into dark bluish green colonies. They produced columnar conidial heads and uniseriate conidiophores. Conidia were globose, bluish green colored and were produced in basipetal succession (Fig. 2c).

Aspergillus tamarii. *Aspergillus tamarii* produced olive green to brown colored colonies. Mycelium is hyaline, septate and branched. Conidiophores were colorless and biseriate in nature. Conidia were spherical and smooth surfaced in nature (Fig. 2d).

Penicillium. Colonies were initially white and became bluish green upon full growth. The margins of the colonies were wavy and concentric rings were visible. On the reverse side of the plate, the colonies were red or pink-tinged at the center and the margin. Conidiophores were either branched or unbranched with metulae at the end. Metulae produced sterigmata in which the conidia were arranged. Conidium was small, uninucleate, globose or ovoid (Fig. 2e).

Fusarium. *Fusarium* produced white fluffy colonies with violet to purple colored mycelium and brown zonation. Sickle-shaped septate macroconidia were observed. Both terminal and intercalary chlamydospores were observed. Chlamydospores were thick and smooth-walled.

Rhizopus. *Rhizopus* produced dark greyish brown fluffy colonies. Simple globose sporangia were observed at the end of sporangiophores. Each sporangiophore arises from the root like a rhizoid. Sporangiospores were dark single-celled and globose to ovoid.

Macrophomina. Colonies were dull white initially and turned to dark brown colored colonies upon time. Mycelium is septateand hyaline at initial stages but turns light brown upon growth. Dark brown colored, oval to spherical microsclerotia were produced by hardening of the fungal mycelium.

Alternaria. *Alternaria* produced dull white to olive-colored colonies with white margins. Conidiophores were simple, septate, smooth walled and pale brown. Conidia were short, obclavate or ovoid with both transverse and longitudinal septa.

This finding is in line with the work of Tsedaley and Adugna (2016), who recovered 110 fungal isolates from three maize varieties and the major fungi observed were *Aspergillus, Fusarium* and *Penicillium*. *Aspergillus*, *Fusarium*, *Penicillium*, *Bipolaris maydis, Alternaria, Cephalosporium*, *Macrophomina, Diplodia, Nigrospora, Botryosphaeria, Cladosporium, Trichoderma, Rhizoctonia* and *Mucor* have been reported from maize seed (Kumar *et al*., 2017). El-Shanshoury *et al*. (2004) isolated and identified eight fungal genera that belonged to *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, *Cladosporium*, *Trichoderma*, *Rhizopus* and *Alternaria* using the standard blotter paper method. Mairevi *et al*. (2012) isolated *Penicillium*, *Aspergillus*, *Alternaria* and *Fusarium* from maize seed. Getachew *et al*. (2018) isolated *Penicillium*, *Aspergillus* and *Fusarium* from maize seeds collected from South and Southwestern Ethiopia.

Relative Density (RD). In terms of Relative Density (RD), *A. niger* was dominant an RD% of 30, followed by *A. flavus* (27%) and *A. fumigatus* (20%) (Fig. 3). *Aspergillus niger*, *A. flavus*, *A. tamarii* and *A. fumigates* were observed in all locations (Fig. 4). *A. oryzae* was observed only in 4 locations. *Fusarium* spp., was observed in all the locations except in Virudhunagar region. *Penicillium* spp., was observed in 5 locations, *Rhizopus* spp., and *Macrophomina* each in 3 locations, and *Alternaria* spp., was observed in 2 locations. A higher number of mycoflora (118) was observed in Tirupur, followed by Dharmapuri (110). In other locations, it ranged from 44-102.

α-Diversity. Shannon-Weiner (H') explains the influence of abundance. As per Edwino Fernando's Ranking of Biodiversity Indices, the Shannon index was less than 1.9 in all locations. In all locations, diversity was very low, hence mycoflora abundance was absent (Table 1). The highest Simpson's index of 0.8019 was observed in the Ariyalur region, representing the highest dominance of mycoflora.

The evenness index values were more than 0.5 in all the locations, which infers that species distribution is even (Table 2). Richness represents the number of species at a region or location. The highest species richness of about 8 was observed in Ariyalur;7 in Madurai and Dindigul; and 6 in Virudhunagar, Namakkal, Tiruppur, Dharmapuri, Oddanchathiram and Perambalur (Fig. 5).

β-Diversity. Beta-diversity is diversity between sites (paired comparison) and it essentially quantifies the number of different communities in the region. Thus, it is the region's absolute number of distinct components (Tuomisto, 2010). Virudhunagar was 50% related to Oddanchathiram and Perambalur. Pairwise comparison between locations gave high dissimilarity since the values were less than 0.5. Low values suggest that there was no spatial variability in the distribution of mycoflora (Table 3).

Genevieve *et al*. (2019) studied the difference in fungal composition between forest stands analyzed with permutational multivariate analysis of variance and beta-diversity partitioning analyses. The most prevalent fungi belonged to the orders Agaricales, Helotiales, and Russulales, while sites from Abitibi-North Témiscamingue's showed the highest OTU (Operational Taxonomic Unit) richness. Kumar *et al*. (2017) reported that the Simpson index of dominance (D), Shannon-Weaver index of diversity (H) and Evenness (E) of *Aspergillus flavus* contributed to fungal diversity. The dominance of mycoflora species varied from place to place and was influenced by various environmental factors, variety, cultivation method and soil, etc.

Fig. 1. Assessment of mycoflora on maize seed by (a) standard blotter paper method, (b,c) Colonization of maize seeds by different fungal genera.

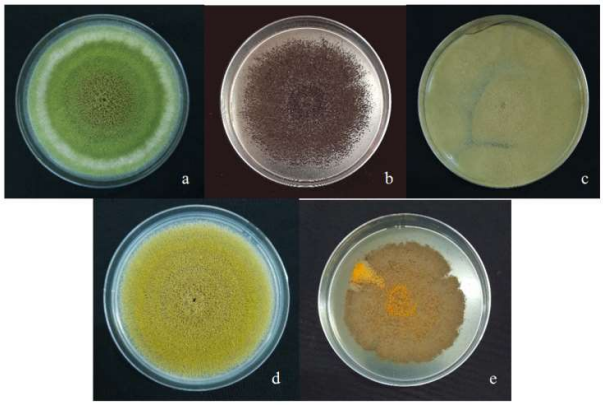

Fig. 2. Pure culture of (a) *Aspergillus flavus,* (b) *Aspergillus niger,* (c) *Aspergillus fumigates,* (d) *Aspergillus tamari,* (e) *Penicillium* on potato dextrose agar.

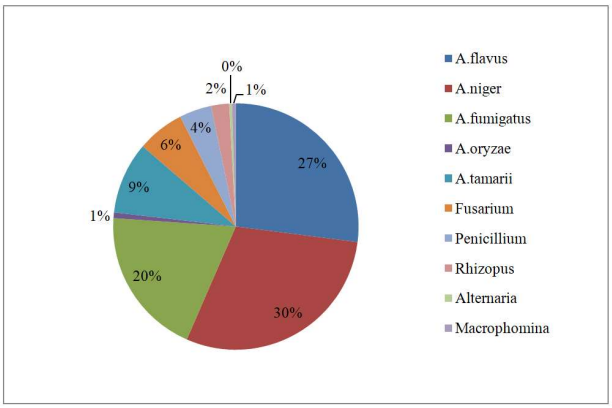

Fig. 3. Relative density of individual fungal species isolated from maize seed.

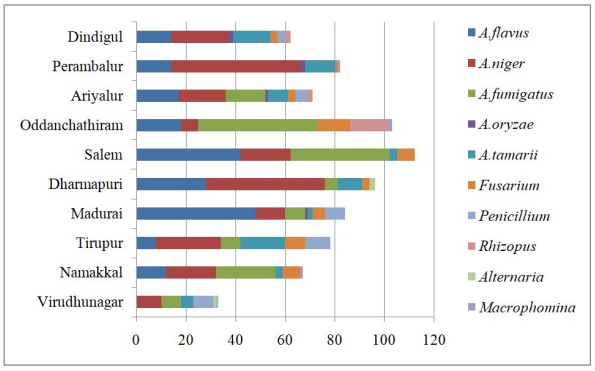

Fig. 4. Distribution of fungal species at different maize growing sites.

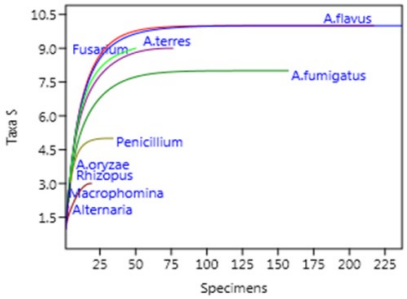

Fig. 5. Fungal species richness and rareness.

Table 1: Edwino Fernando's ranking of Biodiversity Indices.

Relative values	Shannon (H') Index	Evenness (E)
Very high	3.5 and above	0.75 – 1.00
High	3.0 – 3.49	0.50 - 0.74
Moderate	2.5 – 2.99	0.25 – 0.49
Low	2.0 – 2.49	0.15 – 0.24
Very low	1.9 and below	0.05 – 0.14

Table 2: Alpha-diversity indices at different sites.

Particulars	Locations									
	Virudhunagar	Namakkal	Tirupur	Madurai	Dharmapuri	Salem	Oddanchathiram	Ariyalur	Perambalur	Dindigul
Taxa_S	6	6	6	7	6	5	6	8	6	7
Individuals	44	63	118	64	110	88	102	68	82	64
Dominance_D	0.2056	0.2769	0.2537	0.2651	0.3476	0.3076	0.2922	0.1981	0.4536	0.2529
Simpson_1-D	0.7944	0.7231	0.7463	0.7349	0.6524	0.6924	0.7078	0.8019	0.5464	0.7471
Shannon_H	1.6440	1.4490	1.5600	1.5680	1.2590	1.3360	1.4590	1.7690	1.0700	1.5580
Evenness_e^H/S	0.8627	0.7097	0.7932	0.6852	0.5870	0.7610	0.7168	0.7333	0.4859	0.6784

Taxa_S represent the total number of fungal species observed, Individuals represent the total mycoflora (colonies)

Table 3: Beta diversity index between sites.

	Virudhunagar	Namakkal	Tirupur	Madurai	Dharmapuri	Salem	Oddanchathiram	Ariyalur	Perambalur	Dindigul
Virudhunagar	0									
Namakkal	0.33333	0								
Tirupur	0.16667	0.16667	0							
Madurai	0.23077	0.23077	0.076923	0						
Dharmapuri	0.16667	0.16667	0.16667	0.23077	0					
Salem	0.27273	0.090909	0.090909	0.16667	0.090909	0				
Oddanchathiram	0.5	0.16667	0.33333	0.38462	0.33333	0.27273	0			
Ariyalur	0.28571	0.28571	0.14286	0.066667	0.28571	0.23077	0.28571	0		
Perambalur	0.5	0.16667	0.33333	0.23077	0.33333	0.27273	0.33333	0.28571	0	
Dindigul	0.38462	0.38462	0.23077	0.14286	0.38462	0.33333	0.38462	0.066667	0.23077	0

Each value in the cell represents the diversity between two sites

CONCLUSION

The results obtained from this current study are of prime importance to seed certification agencies to prevent post-harvest losses. Colonization of these fungal genera *viz*., *Aspergillus*, *Fusarium*, *Penicillium* etc. renders them unfit for human consumption due to their mycotoxin producing properties. This affects both the economy and the health of the human population.

FUTURE SCOPE

In our study, the predominant mycoflora recorded were the genus *Aspergillus*, *Fusarium* and *Penicillium* which are mostly mycotoxin producing organisms. Similar studies need to be done in different storage buildings because most contamination happens because of bad storage and handling.

Conflict of interest. Nil.

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