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Diversity of Indigenously Isolated *Aspergilli* from Soil of Monoculture Teak Forest

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Abstract: The diversity of *Aspergillus* from soil of teak dominated kusmi forest an Indian subtropical teak forest of Gorakhpur, has been studied and compared with earlier reports. The nine species of *Aspergillus* namely *A. aculeatus*, *A. carbonarius*, *A. deflectus*, *A. fischeri*, *A. niger*, *A. phoenicis*, *A. puniceus*, *A. sparsus* and *A. wentii* were unaffected and uniformly distributed over the time period. There has been substantial loss of 12 species of *Aspergillus* namely *A. carneus*, *A. deflectus*, *A. melleus*, *A. panamensis*, *A. petrakii*, *A. puniceus*, *A. quardilineatus*, *A. rugulosus*, *A. sparsus*, *A. subolivaceus*, *A. sulphureus* and *A. terricola* which were reported in earlier study. A total of four species namely *A. flavor-furcatis*, *A. oryzae*, *A. tubingensis* and *A. zonatus* were supposed to be new because none of literature have reported earlier about those four. The shift in the diversity of *Aspergillus* might be due to changes in the vegetation of the forest over the time.

Key words: Teak vegetation, *Aspergillus*, kusmi forest

INTRODUCTION

Soil is a rich habitat for the growth of microorganisms with fungi as one of the dominant group. Fungi live, multiply and die or disintegrate in the soil and thus they provide rich organic matter, which could be recycled as plant nutrition. Members of the genus *Aspergillus* have been of interest for centuries because of their positive impact as fermenting agents, metabolite producer and because of their negative impact as degraders of agricultural products, their toxicity and their pathogenicity. Recent scientific interests in biogeography and biodiversity, as well as the increasing importance of *Aspergilli* as causal agents of mammalian toxicity and disease have led to an increased need to understand where these fungi occur in nature. Most published work on the global distribution of *Aspergilli* in soils has been descriptive, based on the knowledge and experience of the individual authors (Waksman, 1916; Raper and Fennell, 1965). There have been several studies done on diversity of fungi isolated from different forest soils in India (Kamal and Bhargava, 1972, 1973; Manoharachary, 1977; Rao and Manoharachary, 1981; Rao *et al*., 1984; Reddy *et al*., 1987; Manoharachary *et al*., 1989, 1990; Mohanty and Panda, 1994; Ananda and Sridhar, 2004; Rane and Gandhe, 2006; Tangjang *et al*., 2009), which complies the diversity and distribution of fungi in India, while diversity of filamentous soil fungi from worldwide have

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also studied by several workers for evaluation of different factors responsible for distribution of these fungi (Stanlake, 1977; Klich, 2002; Suhail *et al*., 2007; Donner *et al*., 2009; Kostadinova *et al*., 2009). The diversity of *Aspergillus* from soil of kusmi forest, District Gorakhpur, (26°45'N and 83°24'20"E India) has been extensively studied earlier (Gupta, 1975). The present study provides a comparative account of shift in the diversity of *Aspergillus* from soil of kusmi forest with the earlier reports.

MATERIALS AND METHODS

Study Sites

The site for the present study was an area of about ten hectares of kusmi forest in District Gorakhpur, (26°45'N and 83°24'20"E India). This present study is an serious effort carried between September 2004 to January 2007. The forest is the result of purely planned afforestation scheme with a dominant teak (*Tectona grandis*) vegetation along with certain annual and seasonal vegetation. The soil is alluvial, coarse-sandy and friable in nature. The color of the soil is dark brown to yellow depending on the site and type of vegetation.

Sampling

For sampling a 15 cm deep pits were bored at the site of soil collection by means of soil auger. Half a centimeter soil from the ground surface was removed so as to eliminate litter and other fallen materials. A total of 10 different soil samples covering the entire area were subjected to air dry at room temperature for 5 days prior to isolation of *Aspergillus*.

Culture Conditions

The different isolates of *Aspergillus* from the collected soil samples were isolated by plating method on standard Potato Dextrose Agar (PDA) media. The characterization of different isolates of *Aspergillus* was carried out on Czapek Dox agar Medium (Raper and Fennel, 1965).

Characterization of Aspergillus Isolates

The different isolates of *Aspergillus* were identified and grouped in genera and species based on standard morphological (macroscopic and microscopic) and cultural features (Raper and Fennel, 1965).

Documentation and Computer Analysis

The diversity of *Aspergillus* isolates were statistically evaluated using SPSS window 12.0 version software.

RESULTS

The site for the present study has experienced drastic changes in the vegetation including trees and annuals as reported earlier (Gupta, 1975). The comparative account of the status of tree vegetation over the time is shown in Table 1 with dominant teak vegetation presently, instead of teak there are 15 tree species was found during the time of earlier study carried by Gupta (1975). The present vegetation is enriched with *Delbergia sissoo* and *Shorea robusta* while there has been total loss of 15 tree species which were observed in earlier study (Gupta, 1975). There have been variations in the population of different annuals observed in both studies with significant loss of many of the species and inclusion of different annuals in the present time. The status of 72 annuals has been compared in the

Table 1: Comparative account of the tree vegetation

Tree species	Gupta (1975)	Present study
Adina cordifolia	++	–
Alstonia scholaris	+	–
Artocarpus lackoocha	++	–
Bridelia retusa	+	–
Butea monosperma	++	–
Callicarpa arborea	+	–
Cassia fistula	++	–
Dalbergia sissoo	+++	+
Dillenia pentagyna	+	–
Diospyros exsculpta	+	–
Emblica officinalis	+++	–
Ficus recemosa	++	–
Ficus benghalensis	+	–
Cmelina arborea	+	–
Magnifera indica	++	–
Milusa tomentos	+	–
Shorea robusta	++	+
Tectona grandis	+	++++++++++

Table 2: Comparative account of the annual vegetation

Annual species	(Gupta, 1975)	Present study	Annual species	(Gupta, 1975)	Present study
Achyranthes aspera	+	+	*Ficus heterophylla*	-	+
Aegle marmelos	+	-	*Fimbristylis ovate*	+	+
Ageratum conyzoides	-	+	*Glycosmis arborea*	-	+
Ailanthus excelsa	+	-	*Halarrhina antidyscutrica*	+	-
Alangium salvifolium	+	-	*Hemidesmus indicus*	-	+
Baliospermum montantum	+	-	*Hyptis suaveolens*	+	-
Boerhavia diffusa	+	+	*Indigofera linifolia*	-	+
Butea parviforea	+	-	*Leea chinensis*	+	-
Calastrus xylopyrus	+	-	*Leea macrophylla*	+	+
Calotropis gigantean	+	+	*Lindernia bracheata*	-	+
Capparis zeylanica	+	-	*Litrea glutinosa*	+	-
Caprissa spinarum	+	-	*Maltus philippensis*	+	-
Cardia crenata	+	-	*Martinia diandra*	+	-
Caretia trifolia	+	-	*Meyna laxiflora*	+	+
Casearia elliptica	+	-	*Mimosa himalayana*	+	+
Casearia tomentosa	+	-	*Moghamia tuberose*	+	-
Cassia occidentails	+	+	*Muchia madraspatna*	+	-
Cassia tora	+	+	*Muchia marmelos*	+	-
Cetastrus parrmiculatatus	+	-	*Murrya koenirgii*	+	-
Clematis gouriana	-	+	*Nepeta hindostana*	-	+
Clerodendrum viscosum	-	+	*Peristrophe biclycalata*	+	-
Convolvulus arvensis	-	+	*Pogostemon benghalense*	-	+
Crotalaria medicagenea	-	+	*Polygonum landceolatus*	+	-
Croton roxburghii	+	-	*Rumex denticulatus*	+	-
Cynodon dactylon	-	+	*Scirpus articulatus*	-	+
Cyperus rotundus	-	+	*Smilex prolifera*	-	+
Desmodium pulchelum	+	-	*Solanum nigrum*	-	
Desmostachya bipinnata	-	+	*Tribia nudiflora*	+	-
Dioscorea bulbiflora	+	-	*Tridax procumbens*	-	+
Dioscorea echinata	+	-	*Ventilago madraspatana*	-	+
Dioscorea spinosa	+	-	*Vernonia divergens*	-	+
Dioscoria alternate	+	-	*Woodfordia fructicosa*	-	+
Diospyros melanoxylan	+	-	*Xanthium strumarium*	-	+
Elaeodendrow glavcum	+	-	*Xeromphis spinosa*	+	-
Euphorbia geniculata	+	+	*Zizyphus rotendifolia*	+	-
Evoluvlus numularis	+	+	*Zizyphus xylopyrus*	+	+

present study (Table 2) revealing a significant loss of several annuals over the time. A loss of 37 annuals and inclusion of 22 new annuals were observed in the present study while the status of 13 annuals remained same (Table 3). The changes in the vegetation have direct

Table 3: One-sample statistics analysis of diversity of *Aspergillus* using SPSS software

	Occurrence of *Aspergillus* isolates (% out of total)		Statistics			
Aspergillus sp.	Gupta (1975)	Present study	N	Mean	SD	Std. error mean
A. aculeatus	4.82	5.26	2	5.040	0.31113	0.220
A. amstelodami	0.34	0.96	2	0.650	0.43841	0.310
(E. amstelodami)						
A. awamori	1.27	3.65	2	2.460	1.68291	1.190
A. Candidus	2.95	0.96	2	1.955	1.40714	0.995
A. carbonarius	2.63	2.51	2	2.570	0.08485	0.060
A. carneus	1.78	0.00	2	0.890	1.25865	0.890
A. clavatus	0.20	1.15	2	0.675	0.67175	0.475
A. deflectus	0.11	0.00	2	0.055	0.07778	0.055
A. fischeri	1.38	1.15	2	1.265	0.16263	0.115
(Neosartorya fischeri)						
A. flavipes	5.07	0.96	2	3.015	2.90621	2.055
A. flavor-fercatis	0.00	1.35	2	0.675	0.95459	0.675
A. flavus	16.53	24.04	2	20.285	5.31037	3.755
A. fumigatus	11.27	0.96	2	6.115	7.29027	5.155
A. japonicus	2.84	3.85	2	3.345	0.71418	0.505
A. lanosus	0.31	0.96	2	0.635	0.45962	0.325
A. melleus	0.21	0.00	2	0.105	0.14849	0.105
A. nidulans	4.42	7.69	2	6.055	2.31224	1.635
(E. nidulans)						
A. niger	12.23	12.69	2	12.460	0.32527	0.230
A. ochraceus	5.42	1.54	2	3.480	2.74357	1.940
A. oryzae	0.00	1.35	2	0.675	0.95459	0.675
A. panamensis	0.19	0.00	2	0.095	0.13435	0.095
A. parasiticus	0.11	4.62	2	2.365	3.18905	2.255
A. petrakii	0.29	0.00	2	0.145	0.20506	0.145
A. phoenicis	0.44	0.78	2	0.610	0.24042	0.170
A. puniceus	0.14	0.00	2	0.070	0.09899	0.070
A. quardilineatus	0.37	0.00	2	0.185	0.26163	0.185
(E.quardilineata)						
A. rugulosus	1.01	0.00	2	0.505	0.71418	0.505
(E. rugulosus)						
A. restrictus	0.36	0.96	2	0.660	0.42426	0.300
A. sclerotirum	0.50	1.35	2	0.925	0.60104	0.425
A. sparsus	0.11	0.00	2	0.055	0.07778	0.055
A. subolivaceus	0.34	0.00	2	0.170	0.24042	0.170
A. sulphureus	0.32	0.00	2	0.160	0.22627	0.160
A. sydowi	8.81	0.58	2	4.695	5.81949	4.115
A. tamarii	0.51	5.62	2	3.065	3.61332	2.555
A. terreus	1.63	9.23	2	5.430	5.37401	3.800
A. terricola	0.19	0.00	2	0.095	0.13435	0.095
A. tubingensis	0.00	2.12	2	1.060	1.49907	1.060
A. ustus	0.53	0.96	2	0.745	0.30406	0.215
A. versicolor	9.00	1.34	2	5.170	5.41644	3.830
A. wentii	1.37	0.91	2	1.140	0.32527	0.230
A. zonatus	0.00	1.92	2	0.960	1.35765	0.960

influence in the microflora of soil (Klich, 2002). The *Aspergillus* isolates from different soil samples have been compared with the earlier reports revealing a significant shift in the diversity of *Aspergillus* in terms of number of species. The diversity of *Aspergillus* based on one way sample statistical analysis and test of significant t-test with 5% degree of freedom is provided in Table 3 and 4, respectively. The statistical analysis revealed the uniform presence of nine species namely *A. aculeatus*, *A. carbonarius*, *A. deflectus*, *A. fischeri*, *A. niger*, *A. phoenicis*, *A. puniceus*, *A. sparsus* and *A. wentii* in the present study similar to what has been reported earlier. There has been substantial loss of 12 species of *Aspergillus* namely *A. carneus*, *A. deflectus*, *A. melleus*, *A. panamensis*, *A. petrakii*,

Table 4: One-sample t-test analysis for diversity of *Aspergillus* using SPSS software

	Test value = 0				95% Confidence Interval of the difference		
Aspergillus species	t	df	Sig. (2-tailed)	Mean difference	Lower	Upper	Result
A. aculeatus	22.909	1	0.028	5.04000	2.2446	7.8354	N
A. amstelodami	2.097	1	0.283	0.65000	-3.2889	4.5889	S
A. awamori	2.067	1	0.287	2.46000	-12.6604	17.5804	S
A. Candidus	1.965	1	0.300	1.95500	-10.6877	14.5977	S
A. carbonarius	42.833	1	0.015	2.57000	1.8076	3.3324	N
A. carneus	1.000	1	0.500	0.89000	-10.4185	12.1985	S
A. clavatus	1.421	1	0.390	0.67500	-5.3604	6.7104	S
A. deflectus	1.000	1	0.500	0.05500	-0.6438	0.7538	N
A. fischeri	11.000	1	0.058	1.26500	-0.1962	2.7262	N
A. flavipes	1.467	1	0.381	3.01500	-23.0963	29.1263	S
A. flavor-fercatis	1.000	1	0.500	0.67500	-7.9017	9.2517	S
A. flavus	5.402	1	0.117	20.28500	-27.4268	67.9968	S
A. fumigatus	1.186	1	0.446	6.11500	-59.3855	71.6155	S
A. japonicus	6.624	1	0.095	3.34500	-3.0716	9.7616	S
A. lanosus	1.954	1	0.301	0.63500	-3.4945	4.7645	S
A. melleus	1.000	1	0.500	0.10500	-1.2292	1.4392	S
A. nidulans	3.703	1	0.168	6.05500	-14.7196	26.8296	S
A. niger	54.174	1	0.012	12.46000	9.5376	15.3824	N
A. ochraceus	1.794	1	0.324	3.48000	-21.1700	28.1300	S
A. oryzae	1.000	1	0.500	0.67500	-7.9017	9.2517	S
A. panamensis	1.000	1	0.500	0.09500	-1.1121	1.3021	S
A. parasiticus	1.049	1	0.485	2.36500	-26.2875	31.0175	S
A. petrakii	1.000	1	0.500	0.14500	-1.6974	1.9874	S
A. phoenicis	3.588	1	0.173	0.61000	-1.5501	2.7701	N
A. puniceus	1.000	1	0.500	0.07000	-0.8194	.9594	N
A. quardilineatus	1.000	1	0.500	0.18500	-2.1656	2.5356	S
A. rugulosus	1.000	1	0.500	0.50500	-5.9116	6.9216	S
A. restrictus	2.200	1	0.272	0.66000	-3.1519	4.4719	S
A. sclerotirum	2.176	1	0.274	0.92500	-4.4751	6.3251	S
A. sparsus	1.000	1	0.500	0.05500	-0.6438	0.7538	N
A. subolivaceus	1.000	1	0.500	0.17000	-1.9901	2.3301	S
A. sulphureus	1.000	1	0.500	0.16000	-1.8730	2.1930	S
A. sydowi	1.141	1	0.458	4.69500	-47.5910	56.9810	S
A. tamarii	1.200	1	0.442	3.06500	-29.3994	35.5294	S
A. terreus	1.429	1	0.389	5.43000	-42.8536	53.7136	S
A. terricola	1.000	1	0.500	0.09500	-1.1121	1.3021	S
A. tubingensis	1.000	1	0.500	1.06000	-12.4086	14.5286	S
A. ustus	3.465	1	0.179	0.74500	-1.9868	3.4768	S
A. versicolor	1.350	1	0.406	5.17000	-43.4948	53.8348	S
A. wentii	4.957	1	0.127	1.14000	-1.7824	4.0624	N
A. zonatus	1.000	1	0.500	0.96000	-11.2380	13.1580	S

A. puniceus, *A. quardilineatus*, *A. rugulosus*, *A. sparsus*, *A. subolivaceus*, *A. sulphureus* and *A. terricola* when compared with earlier reports (Table 4). The diversity of *Aspergillus* in the present study further revealed the occurrence of four species namely *A. flavor-furcatis*, *A. oryzae*, *A. tubingensis* and *A. zonatus* not reported earlier (Gupta, 1975). The diversity of *Aspergillus* in terms of number of strains of different species revealed *A. flavus* to be the dominant species as compared to *A. niger* in the earlier report (Gupta, 1975) as shown in Table 4.

DISCUSSION

The comparative account of diversity of *Aspergillus* isolated from soil of same forest reveals the influence of vegetation in distribution of micorflora of soil. The monoculture teak

vegetation of the forest during the recent time resulted in shift in the diversity of *Aspergillus* as this group of fungi being saprotrophs is directly influenced by litter and humus composition of the soil (Ananda and Sridhar, 2004). Further it has been reported that there exists strong relation between litter and humus composition with type of vegetation on the same soil (Bardgett and Shine, 1999; Klich, 2002; Samson and Hong, 2006). The present study clearly indicates the shift in the diversity of *Aspergillus* isolated from soil samples of kusmi forest as compared to earlier studies revealing the direct influence of vegetation on the population of microflora of a particular soil as suggested in earlier reports (Mohanty and Panda, 1994; Rane and Gandhe, 2006; Tangjang *et al*., 2009). The monoculture teak vegetation of kusmi forest in the recent past has resulted in changes in the diversity of *Aspergillus* with elimination of some species and occurrence of some new species and also in terms of frequency of occurrence of different species when compared with earlier study carried out at same site. It is quite obvious that since there has been shift in the vegetation of study site, the soil also undergoes changes in the composition of microbial flora as a result of which a drastic changes in the *Aspergillus* population is observed (Klich, 2002).

The present investigation on comparative account of the biodiversity of indigenous isolates of *Aspergillus* provides interesting out comes as discussed above. Loss of some species *Aspergillus* from this region is a serious issue concerning with conservation of microbes diversity, such type of losses can't be recovered. The change in composition of diversity of *Aspergilli* especially in occurrence of frequency of species and their shift may cause the harm to other microflora as well as microfauna which also need to be investigated further. The present study is a basic research in compositional shifting of *Aspergilli* in respect of change in higher vegetations, which certainly open so many aspects to think and work in the direction of microbial conservation and typing for further biological and biotechnological applications.

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