Morphological and Molecular Identification and Categorization of *Aspergillus* Isolates Associated with Different Crops from Delhi Market, India

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Abstract

Aspergillus species infecting fruits (Apple & Brinjal) and crop seeds (Maize, wheat, beans, groundnut, paddy and black gram) collected from the market of Delhi during 2008 were identified by using morphological and molecular methods. Sixty-five diseased specimens of fruits and crops were collected from the market and isolations of the pathogens were made. Twenty isolates of *Aspergillus* were separated on the basis of genus characters. The isolates were further categorised into five different species based on their colony characters, viz. colony growth, color and texture and microscopic observations,i.e. conidial head, conidiophore, vesicle, sterigmata and conidia. Random amplified polymorphic DNA (RAPD) fingerprints using six arbitrary 10 mers primers clearly separated the species. Morphological grouping and speciation matched with the molecular grouping for most of the isolates. The random primer OPB11 gave reproducible and very stable result for strain delineation of five examined pathogenic *Aspergillus* species (*A. niger, A. flavus, A. parasiticus, A. terreus* and *A. ochraceus)* in comparison to other primers and this primer was used to develop species specific marker to identify *Aspergillus flavus* as it gave monomorphic band for all the *A. flavus* isolates studied.

Key words: *Aspergillus*; Genetic diversity; RAPD; Speciation

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INTRODUCTION

Aspergillus is an important genus containing some toxigenic fungi that cause postharvest spoilage. Ayalew (2010) reported that *Aspergillus* is found in association with barley, maize, teff, wheat and sorghum and produced mycotoxins which are harmful to consumer health. Mohammed et al. (2016) isolated fungi from groundnut seeds and reported that *Aspergillus flavus* was the dominant species followed by *Aspergillus parasiticus* in infecting groundnut in eastern Ethiopia. *Aspergillus niger* causes black mold disease on grapes, onions and peanuts and it is a common contaminant of food (Sharma, 2012). It decreases percent seed germination during storage by invasion of the embryos and increases fatty acids by deteriorating seeds (Agrios, 2005). *A. flavus* and *A. niger* showed the highest rate of occurrence in the marketed papaya fruits in Southwest Nigeria (Baiyewu et al., 2007). Aflatoxin producing *Aspergillus* species, viz, *A. flavus* and *A. parasiticus* can infect grains from pre-harvest stage in the field to postharvest in the store (Bandyopadhyay et al., 2005). They occur in and upon a great variety of substrata, being considered as common food spoilage fungi (Pitt and Hocking, 1997).

Aspergillus taxonomy is complex and ever evolving. The genus is easily identified by its characteristic conidiophore but species identification and differentiation is complex, for it is traditionally based on a range of morphological features (Rodrigues et al., 2007). Although molecular methods continue to improve and become more rapidly available, microscopic and cultural (macroscopic) techniques remain commonly used and essential tools for identification of *Aspergillus* species (McCleny, 2005).

Diba et al. (2007) reported that the macroscopic characteristics including colony diameter, color, exudates, colony texture and the microscopic characteristics (conidial heads, stipes, color, length, vesicles shape and seriation, phialide, metula

covering, conidial size, shape and roughness) are the remarkable features for species identification. The sporangium or conidial heads are used to distinguish the different groups of *Aspergillus* species. These heads are formed by conidiophores, vesicle, and a series of primary sterigmata, followed by a second series of secondary sterigmata of which the conidia or spores sprout (Soledad et al., 2005).

Mitchel et al. (1994) developed polymerase chain reaction (PCR) primers to identify *Aspergillus fumigatus* at low stringency**.** Peter et al**. (**2002**)** studied SDS-PAGE and RAPD to identify *A. fumigatus*, *A. niger, A. terreus* and *Aspergillus ustus*, and reported that RAPD analysis showed a high degree of discriminatory power.

Aspergillus tubingensis and *A. niger* have a high morphological similarity, and *A. tubingensis* was considered as a subspecies of *A. niger* but the utilization of molecular methods allowed a better distinction among the species (Accensi et al., 2001). RAPD using the RI08 primer was successful in species differentiation of *A. flavus*, *A. fumigatus* and *A. niger* (Novak et al*.,* 2004; Raclavskya*,* 2006). Identification of the most common toxigenic *Aspergillus* species associated with fruit and other important crop species remained problematic due to the variability in the phenotypic and genotypic characters. The new taxonomies are based on a poly-phasic approach using phenotypical characters, like macro-and micro-morphology together with multi-gene DNA sequence (Robert et al*.,* 2006).

Therefore, the objectives of this study were to investigate the diversity and distribution of *Aspergillus* species associated with different crops and identify the *Aspergillus* species involved based on morphological and molecular diagnostic methods.

MATERIALS AND METHODS

The study on *Aspergillus* species existing in the diseased fruits and seeds collected from the market of Delhi was undertaken during 2008 (Table 1). Sixty-five diseased specimens were collected in polythene bags and incubated on moist cotton in petriplates (28 $\pm$ 2 ^{o}C) for 3-7 days and fruiting structure of the fungi were collected and isolated on potato dextrose agar (PDA) medium. Based on genus characters, twenty *Aspergillus* species isolates were separated and further identified to species level

Macroscopic studies

Spores from individual colonies of *Aspergillus* species grown on PDA were transferred to fresh PDA plates using a sterile needle, and then streak isolated using a sterile loop to obtain separate individual colonies. Plates were incubated at 30 °C for 72 h, and then small pieces of agar containing hyphal tips were transferred to Czapek dox medium prepared according to Horn et al. (1996).

The major and outstanding macroscopic features like colony growth, color and texture were studied for species identification (Diba et al., 2007). Fungal growth was measured at the reverse side of the colonies with the scale in centimeters at intervals of 24 hours starting from the third day of incubation. Further, all isolated *Aspergillus* species were identified to the species level using taxonomic systems by Klich (2002). Data collected on radial growth of the *Aspergillus* isolates were analyzed by SAS statistical package (SAS 9.1.3, 2009).

Table 1. Plant sources for isolation and identification of different *Aspergillus* species/isolates

Sample number	Source	
	Common name	Scientific name
1	Groundnut seed	*Arachis hypogaea*
2	Maize seed	*Zea mays*
3	Maize seed	*Zea mays*
4	Groundnut seed	*Arachis hypogaea*
5	Wheat seed	*Triticum aestivum*
6	Soyabean seed	*Glycine max*
7	Groundnut seed	*Arachis hypogaea*
8	Apple fruit	*Malus pumila*
9	Mushroom pileus	*Agaricus bitorquis*
10	Apple fruit	*Malus pumila*
11	Wheat seed	*Triticum aestivum*
12	Soyabean seed	*Glycine max*
13	Bean pod	*Dolichos lablab*
14	Soyabean seed	*Glycine max*
15	Black gram seed	*Vigna mungo*
16	Bean pod	*Phaseolus vulgaris*
17	Paddy seed (rice)	*Oryza sativa*
18	Wheat seed	*Triticum aestivum*
19	Wheat seed	*Triticum aestivum*
20	Brinjal fruit	*Solanum melangena*

Microscopic studies

Microscopic features of isolates, *viz.* conidial head, vesicle size, sterigmata, conidiophore wall, size and wall of conidia were taken as parameters for differentiation of species. The conidial head shape was observed directly from the tube under microscope and pictures were taken under X100 magnification. Microscopic slides were prepared and the shape, fertile area and size of the vesicle, bi/uni series of sterigmata, conidiophore roughness, size and wall of conidia were recorded. The photomicrographs of the above characters were taken using Olympus digital camera (Olympus cx41 model, Japan). Conidia of the fungal species were drawn using camera lucida and measured in µm (Model: E.LEITZ, Wetzlar, Germany).

Genetic analysis

Fungal cultures were sub-cultured onto 100 ml Erlenmeyer-flasks containing 25 ml. (per litter: 1 g K_2HPO_4; Czapek concentrate, 10 ml; yeast extract, 5 g and sucrose, 200 g) for ten days using a rotator shaker (27°C at 150 rpm). Genomic DNA for RAPD analysis was extracted from 200-300 mg frozen mycelium of *Aspergillus* species based on Cetyltrimethyl ammonium bromide (CTAB) extraction method of Raeder and Broda (1985) with partial modification. The DNA concentration and purity of the samples was determined with Nano Drop Spectrophotometer. PCR conditions and separation of RAPD-PCR fragments were carried out according to Messner et al. (1994). The PCR protocol was standardized by changing the variables like template DNA, Taq DNA polymerase and $MgCl_2$. The optimum amplification was obtained by using a reaction mix having 50 ng template DNA, 5 unit Taq polymerase, 50 mM $MgCl_2$, 10 µM primer and 10 µM dNTPs in reaction volume of 25 µl. Eight random 10 mer Operon primers from set of OPA, OPB, OPZ and another primer R108 were screened. Of these, six primers were selected for RAPD analysis based on their reproducibility and score able polymorphic bands obtained (Table 2).

Table 2**.** Primers used for RAPD analysis of different isolates of *Aspergillus* species

Number	**Primer**	**Sequence 5' to 3'**
1	OPB2	TGATCCCTGG
2	OPB5	TGCGCCCTTC
3	OPB7	GGTGACGCAG
4	OPB11	GTAGACCCGT
5	OPB17	AGGGAACGAG
6	OPB18	CCACAGCAGT
7	RI08	GTATTGCCCT
8	OPZ19	GTGCGAGCAA
9	OPA20	GTTGCGATCC

RAPD profiles were scored visually by comparing RAPD amplification profiles and scoring the presence or absence of each band in each profile according to Halmschlager et al. (1994). The bands on agarose gel electrophoresis were visualized and manually scored by hand to a two - discrete - character - matrix (0 and 1 for absence and presence of RAPD - markers). All amplifications were repeated at least twice and only reproducible bands were considered for analysis. The data obtained from RAPD analysis were subjected to un-weighed pair – group method for arithmetic average analysis (UPGMA) to generate a dendrogram using average linkage procedure.

RESULTS AND DISCUSSION

Twenty *Aspergillus* species isolates were obtained from the diseased fruits and crop seeds collected from the market randomly (Table 1).

Macroscopic studies

On the basis of growth on PDA, the isolates were categorized in to three groups. Group I consisted of eight isolates (isolates: 4, 5, 9, 10, 12, 14, 15, and 16) that were slow growing (less than 4.0 cm in diameter/ seven days). Group II had seven isolates (isolates 1, 2, 3, 6, 8, 11 and 18), which were moderately growing (4.0 – 5.0 cm in diameter/seven days). Group III had five isolates (isolates 7, 13, 17, 19 and 20) that were rapidly growing (more than 5.0 cm in diameter/seven days) (Table 3; Fig. 1).

According to colony color and texture, all the 20 isolates were classified into five different groups (Fig. 2). In the first group of isolates, the mycelium was surface and plane in most of the isolates (nos. 6, 7 and 17) but in one isolate it was radially furrowed (no. 1). The color of the colony of all the isolates within this group was yellow green when young and turned to jade green with age. The second group consists of isolates, the mycelium of which was commonly surface and plane (nos. 5, 11, and 19), slightly raised at the center (no. 5) and slightly furrowed (no. 20). Colony color was yellowish green when young and turned to brownish green with age. Colony texture of group three isolates was surface, plane and slightly raised at the center (no. 12) and occasionally radially furrowed (no. 16) with yellow brown when young later turned to dull brown color. Isolates in group four had mycelium, which is submerged and wrinkled, yellow when young and turned to orange brown with age. Isolates within the fifth group had velvety mycelium, which is plane and radially furrowed with darkest brown to carbonaceous black in color.

Table 3. Colony characteristics of the 20 isolates of *Aspergillus* species

Isolate number	Growth (cm)	Texture	Color
1	4.5	Surface, furrowed	Yellow-brown-green
2	4.0	Velvety, furrowed	Darkest brown
3	4.0	Velvety, furrowed	Carbonaceous. Black
4	3.0	Floccose	Light yellow
5	4.0	Raised, Plane	
6	5.5	Surface, furrowed	Yellow-brown-green
7	6.0	Surface, Plane	Yellowish brown
8	4.2	Velvety, furrowed	Darkest brown
9	2.8	Surface, submerged & furrowed	Brown - woody brown
10	2.6	Surface, submerged & furrowed	Yellow brown
11	4.0	Surface, plane	Yellow - dark green
12	3.0	Raised, Plane	Yellow brown
13	5.0	Velvety, furrowed	Darkest brown
14	3.0	Surface, & submerged, slightly	Brown
15	3.2	Raised, furrowed	Dirty brown
16	3.5	Raised, furrowed	Dull yellow brown
17	5.8	Slightly raised, plane	Yellowish green – brown green
18	2.8	Surface, submerged & furrowed	Brown
19	5.2	Raised, Plane	Bright yellow.-dark brown green
20	5.2	Surface & furrowed	Yellow Green – dark brown green

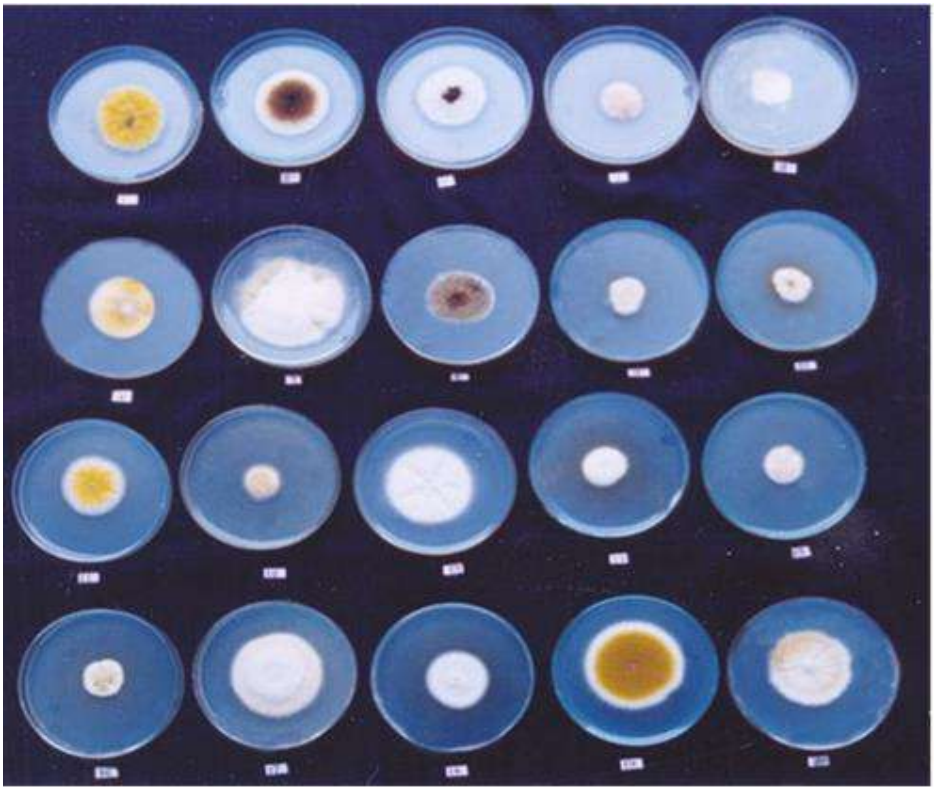
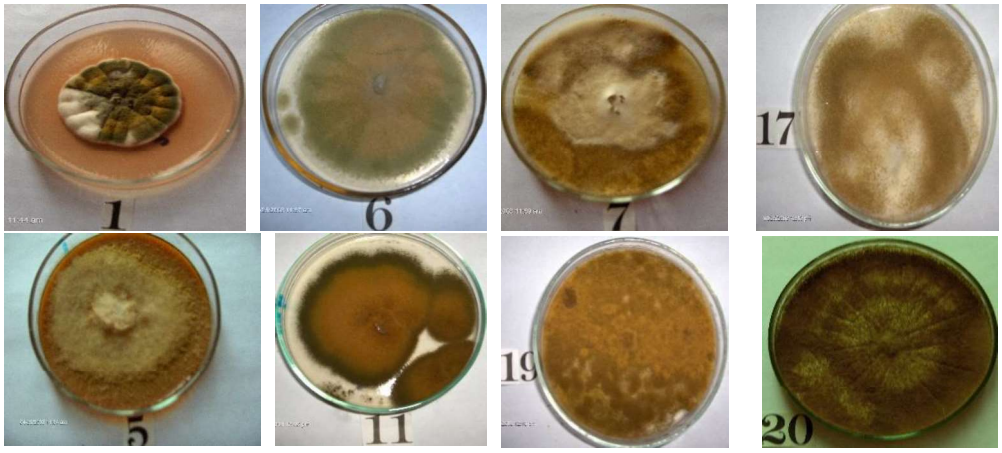


Figure 1. Colony growth of 20 different isolates of *Asperigillus* species in 7days old cultures.

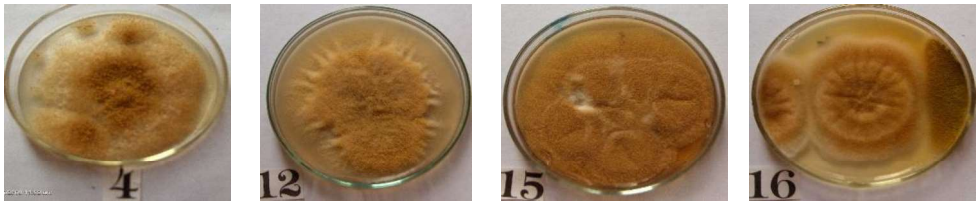
Microscopic studies

In most of the isolates, the shape of conidial head was radiate usually splitting in to many but in group three isolates (nos. 4, 12, 15, and 16), the conidial heads were globose and in group four isolates (nos. 9, 10, 14 and 18) the conidial heads were long columnar (Fig. 3).

The conidiophore wall was rough only in isolates 1, 6, 7 and 17, whereas the remaining isolates of all *Aspergillus* species had smooth wall. The vesicle shape of all the isolates was more or less globose (Fig, 4). The size of the vesicle ranged from 10 - 15 to 51 – 75µm in diameter in different groups. In the isolates 1, 9, 10, 14, 17 and 18, the vesicle was not entirely fertile (Table 4; Fig. 4).

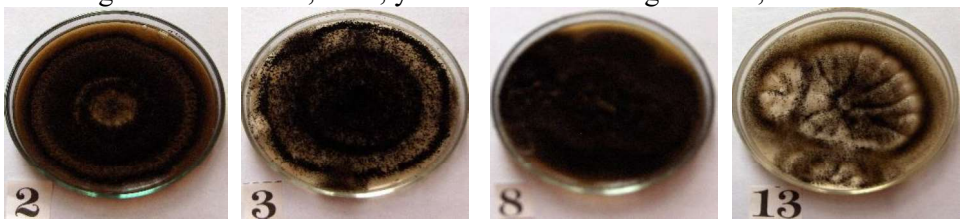


Surface / raised, plane/ furrowed, moderate, yellow green – brownish green



Raised, plane/furrowed, slow, yellow brown - dull brown

Submerged and wrinkled, slow, yellow brown – orange brown,



Velvety, furrowed, moderate, dark brown- black

Figure 2. Colony color and texture of the 20 isolates of *Aspergillus* species (3 weeks old)

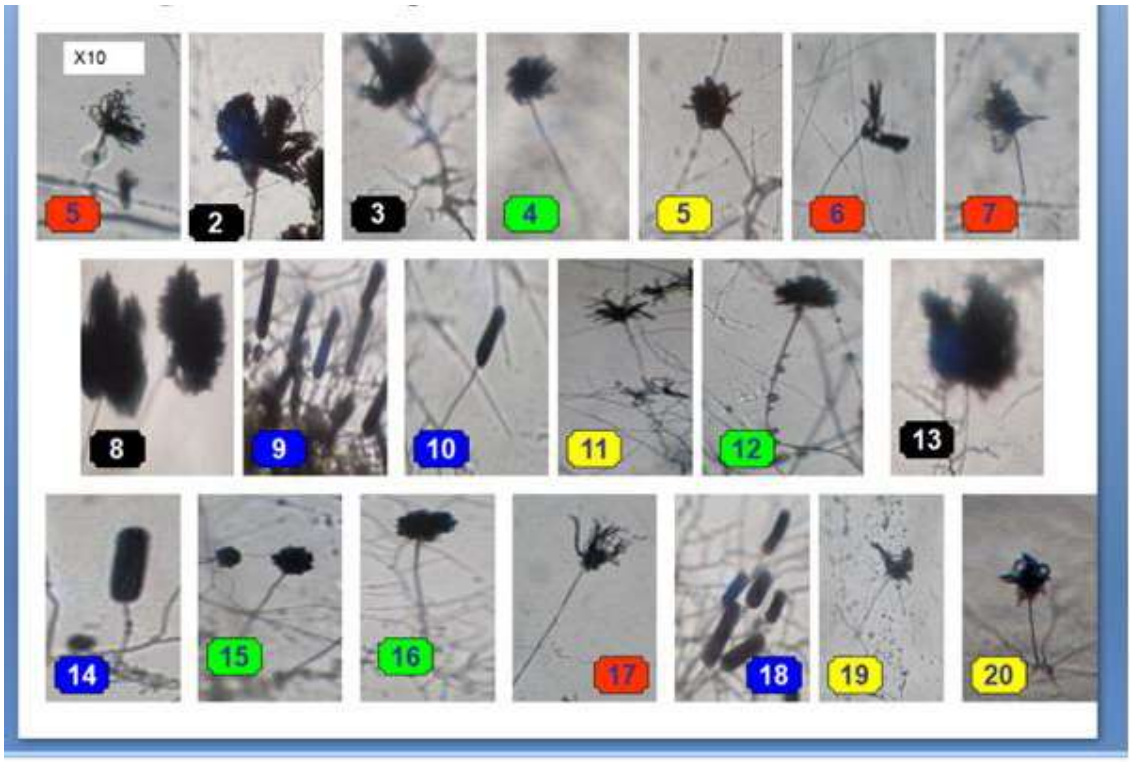


Figure 3. Size and shape of the conidial heads of 20 isolates

The arrangement of sterigmata was in two series in most of the isolates (nos. 1, 2, 3, 4, 6, 8, 9, 10, 12, 13, 14, 15, 16 and 18) but it was single series in some isolates (nos. 5, 7, 11, 17, 19 and 20). In group one isolates, sterigmata was uniserate for isolates 7 and 17, whereas isolates 1 and 6 showed biseriate sterigmata. Sterigmata in group two isolates (5, 11, 19, and 20) were in one series only measuring 10 – 13µm in length. All the isolates of group three (nos. 4, 12, 15 and 16) and four (nos. 9, 10, 14 and 18) showed biseriate sterigmata. In group five isolates (nos 2, 3, 8 and 13), the primary sterigmata was double to the secondary sterigmata in size (Table 4).

The conidial walls were smooth for isolates 4, 9, 10, 12, 14, 15, 16 and 18, whereas the remaining isolates were rough walled (Fig. 5). The diameter of conidia of group 1, 2, 3, 4 and 5 were 6 - 8, 8 - 10, 3 - 4, 2 - 3, and 4 - 6µm, respectively (Table 4; Figs. 5 and 6).

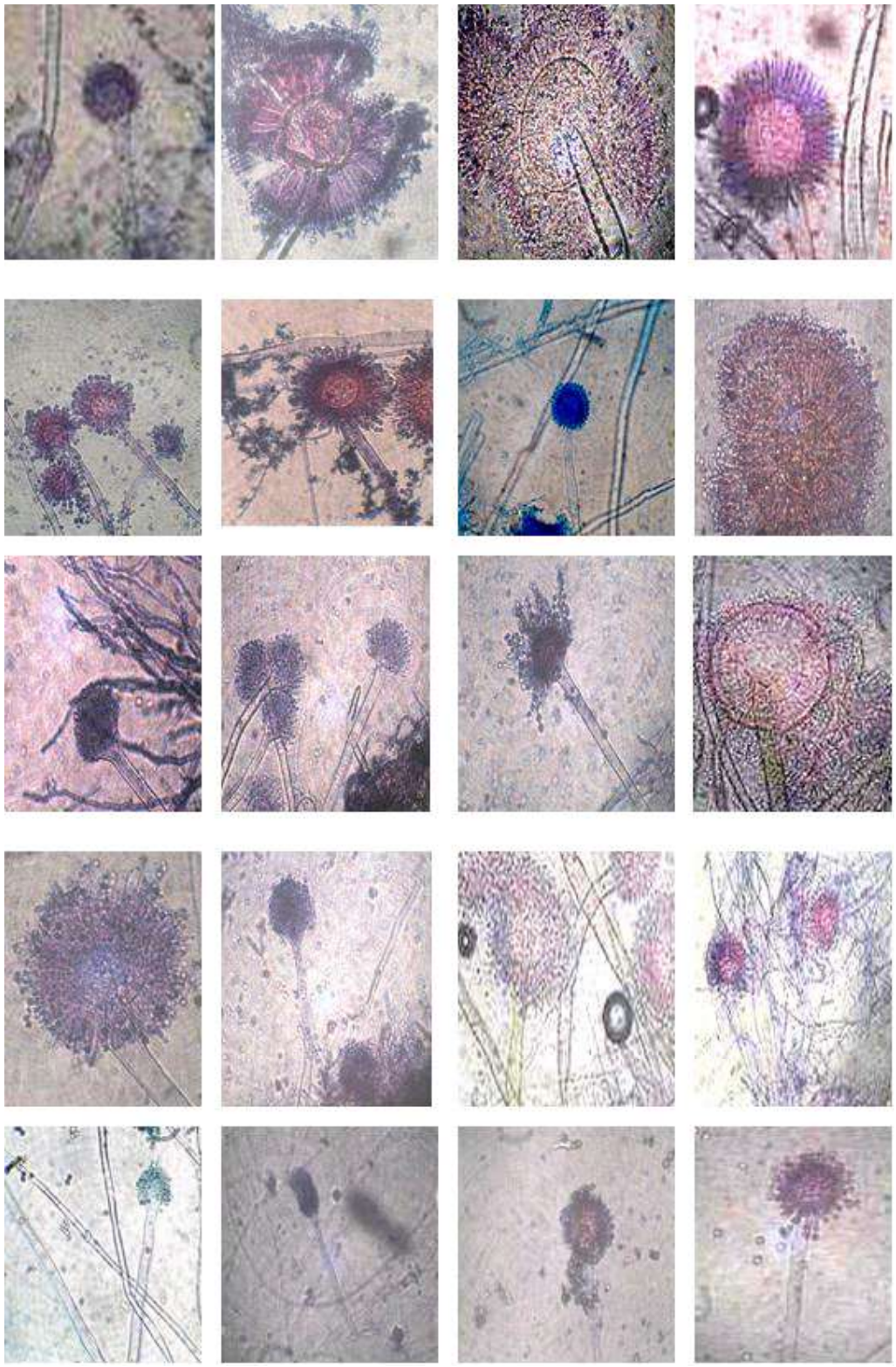


Figure. 4. Vesicle, Sterigmata and Conidiophore characters of 20 isolates *Aspergillus* species (X400)

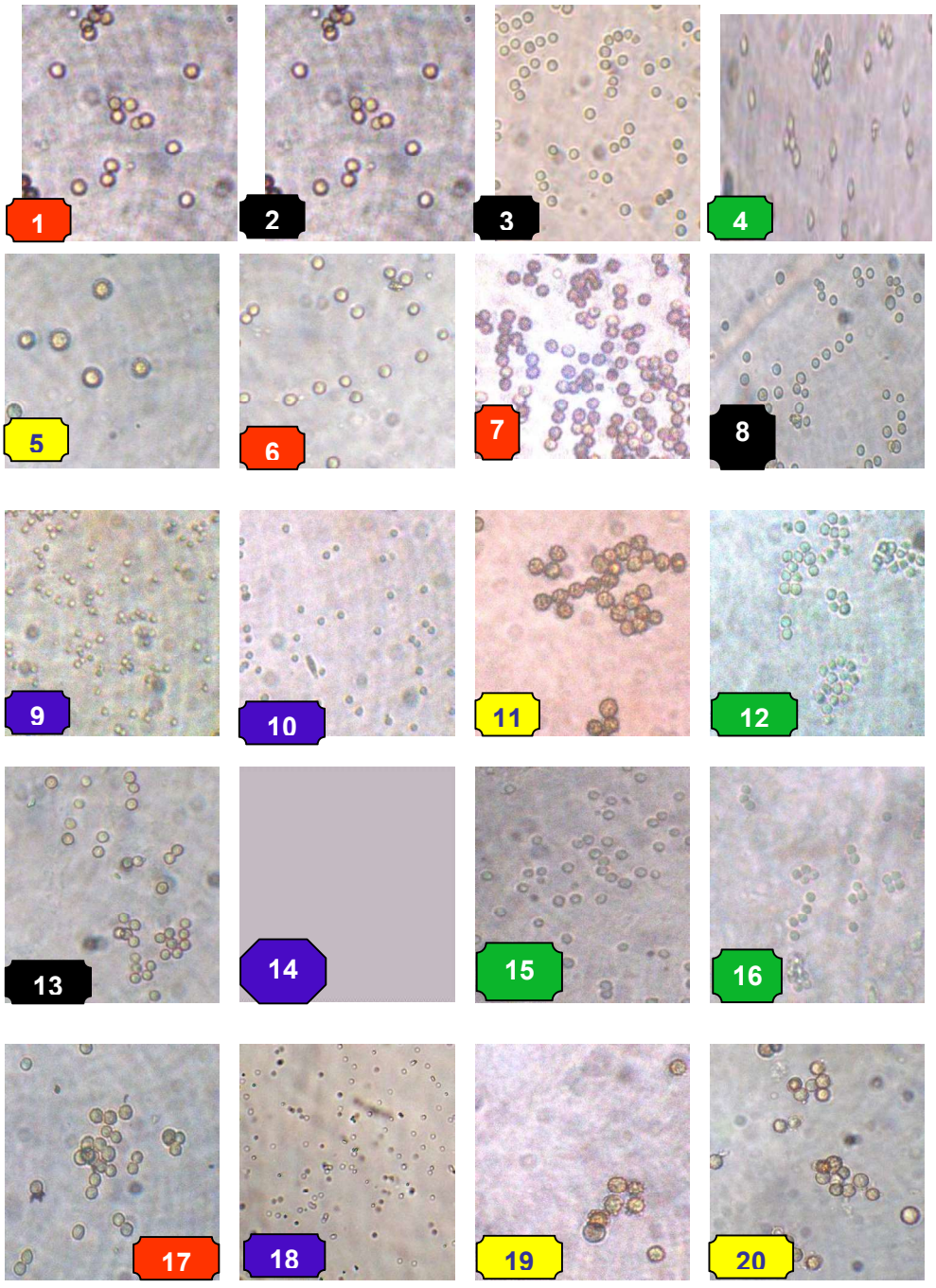


Figure 5. Size and texture of the conidia of 20 different isolates of *Aspergillus* species (X400)

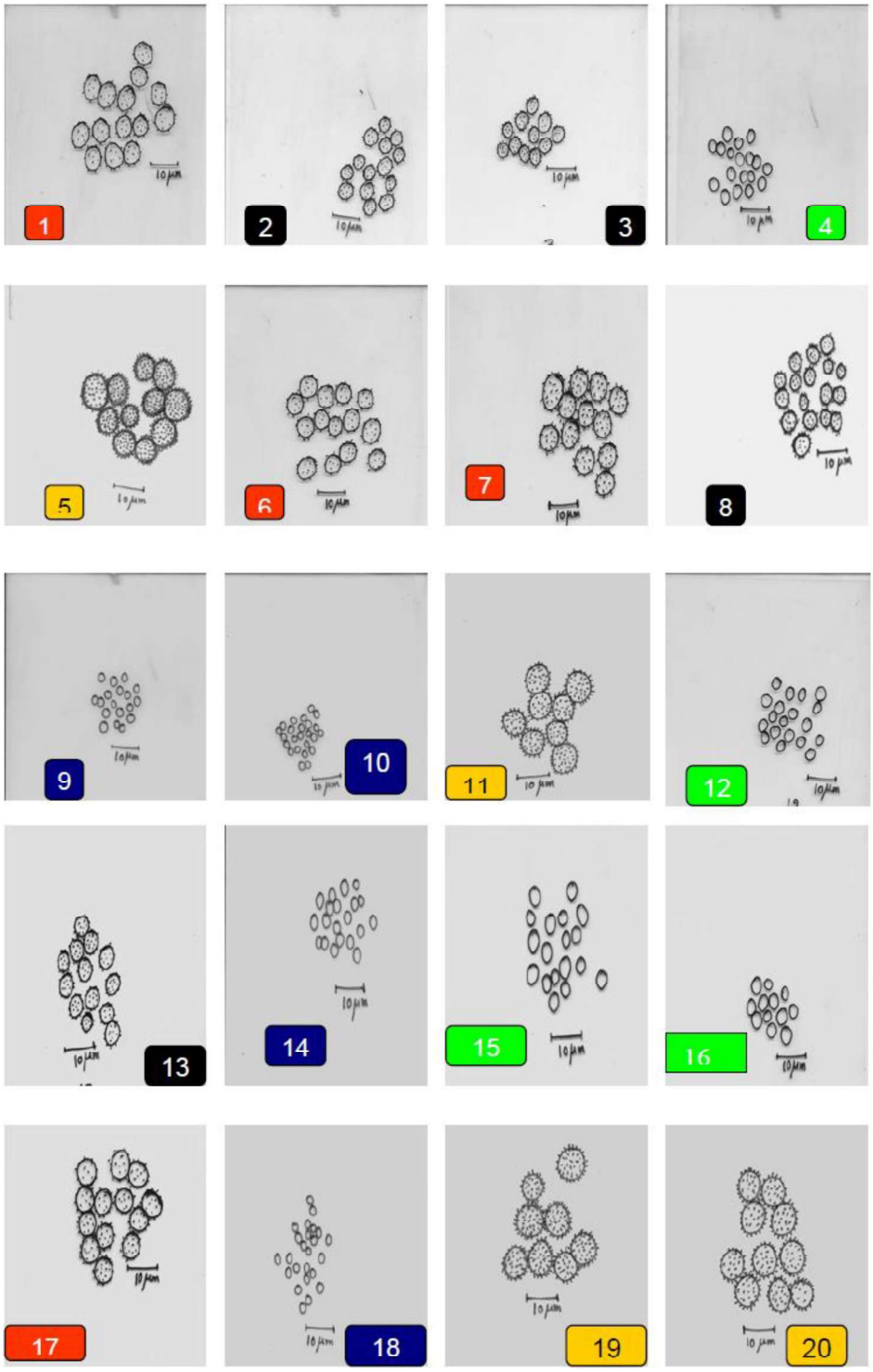


Figure 6. Camera lucida drawings of conidia of the 20 isolates of *Aspergillus* species

Table 4**.** Conidial stage characteristics of the 20 isolates of *Aspergillus* species

Isolate number	Conidial Head	Vesicle		Sterigmata (μm)		CP Wall	Conidia	
		Size (μm)	Fertile area	Uniserate	Biserate		Size (μm)	wall
1	Radiate	28-30	NFC	10-18	11.0	Rough	6.0-7.5	rough
2	Radiate	55-75	FC	35.0	15.0	Smooth	4.0-6.0	"
3	Radiate	58-70	"	15.0	7.0	"	4.0-6.0	"
4	Globose	40-50	"	10.0	14.0	"	3.5-4.0	smooth
5	Radiate	18-25	"	12.0	---	"	6.0-8.5	rough
6	Radiate	27-35	"	13-15	15-20	Rough	6.5-8.0	"
7	Radiate	29-33	"	13-15	---	"	6.0-8.0	"
8	Radiate	51-75	"	40-45	20.0	smooth	4.0-6.0	"
9	LC	11-14	NFC	5.0	10.0	"	2.5-3.0	smooth
10	LC	10-15	" "	7.0	7.0	"	2.5-3.0	"
11	Radiate	20-25	FC	13.0	---	"	8.0-9.0	rough
12	Globose	38-45	"	10.0	20.0	"	3.5-4.0	smooth
13	Radiate	52-65	"	16.0	7.0	"	4.0-6.0	rough
14	LC	12-15	NFC	8.0	6.0	"	2.0-3.0	smooth
15	Globose	38-50	FC	5.0	13.0	"	3.5-4.0	"
16	Globose	40-45	"	7.0	14.0	"	3.0-4.0	"
17	Radiate	30-33	NFC	8-10	---	rough	7.5-8.0	rough
18	LC	13-14	"	7.0	7.0	smooth	2.5-3.0	smooth
19	Radiate	22-25	FC	10.0	---	"	9.0-10	rough
20	Radiate	20-25	"	10.0	---	"	9.0-10	rough

Considering all the above morphological characters, the isolates were categorized into five different groups based on the key below:

***Aspergillus* species identification key made based on morphology (**Raper and Fennell, 1965)

1. Rapid - moderately growing, yellow green when young, conidia definitely echinulate.

A. Colony turned to jade green with age, sterigmata uni / biseriate, big vesicle size (27-35μm) and fertile area entire or not. Conidiophore (CP) wall rough and conidia 6-8μm**……..Group 1**

AA . Colony turned to dark brownish green with age, sterigmata uniseriate, smaller vesicle size (18-25μm), fertile are entire. Conidiophore wall smooth, conidia 8–10μm…….**Group 2**

2. Slow growing, yellow brown when young, conidial wall smooth

A. Colony turned to dull brown with age, head globose, bigger vesicle size (38-50 µm), conidia 3-4µm……..**Group 3**

AA. Colony turned to orange brown with age, head columnar, smaller vesicle size (10 – 15µm), conidia 2-3µm……**Group 4**

3. Moderately growing, Colony darkest brown to carbonaceous black, head radiate, very big vesicle (51 – 75 µm), biseriate (primary sterigmata is double in length to secondary sterigmata), conidia 4-6µm. ……...**Group 5**

The morphological characters of all five groups observed in the study were compared with previous literatures (Raper & Fennel, 1965: Collier et al., 1998; Larone, 2002; Diba et al., 2007; Rodrigues et al., 2007). Accordingly, Group I was placed under *Aspergillus flvaus* (isolates 1, 6, 7 and 17), Group II as *A. parasiticus* (isolates 5, 11, 19 and 20), Group III as *A. ochraceus* (isolates 4, 12, 15 and 16), Group IV as *A. terreus* (isolates 9, 10, 14 and 18) and Group V as *A. niger* (isolates 2, 3, 8 and 13) (Table 5).

All the 20 isolates were deposited at the Indian Type Culture Collection (ITCC) and the accession numbers are given (Table 6).

Table 5. Speciation of groups of the 20 isolates of *Aspergillus* species

Group number	Species	Isolate number
1	*Aspergillus flavus*	1, 6, 7 and 17
2	*Aspergillus parasiticus*	5, 11, 19 and 20
3	*Aspergillus ochraceus*	4, 12, 15 and 16
4	*Aspergillus terreus*	9, 10, 14 and 18
5	*Aspergillus niger*	2, 3, 8 and 13

Table 6. Deposition of 20 isolates of *Aspergillus* species at Indian Type Culture Collection

Isolate	Species	ITCC
1	*A. flavus*	6347
2	*A. niger*	6348
3	*A. niger*	6349
4	*A. ochraceus*	6350
5	*A. parasiticus*	6351
6	*A. flavus*	6352
7	*A. flavus*	6353
8	*A. niger*	6354
9	*A. terreus*	6355
10	*A. terreus*	6356
11	*A. parasiticus*	6357
12	*A. ochraceus*	6358
13	*A. niger*	6359
14	*A. terreus*	6360
15	*A. ochraceus*	6361
16	*A. ochraceus*	6362
17	*A. flavus*	6363
18	*A. terreus*	6364
19	*A. parasiticus*	6365
20	*A. parasiticus*	6366

Molecular analysis

A total number of 329 bands were produced for the *Aspergillus* isolates and all of them were polymorphic. The maximum number of bands (86) was obtained with primer OPB11. The size of fragments obtained ranged from 0.5 to 4kb (Fig. 7). The dendrogram showed that the isolates representing various *Aspergillus* species are different.

Isolates formed two main clusters with all the six primers tested (Fig. 8). The cluster analysis separated one of the isolates (Isolate no. 5) of *A. parasiticus* from all other isolates. The dendrogram obtained separated *A. niger* isolates (Isolates no. 2, 3, 8 and 13) from all the other isolates and revealed more than 75% similarity among themselves. Isolate 1 was separated from other isolates (Isolates 6, 7 and 17), which were in same group under *A. flavus* according to morphological identification. These *Aspergillus flavus* isolates formed one cluster at 40% similarity. Isolates 9, 10, and 14 formed another cluster at 48% similarity, whereas isolate 18 was separated from

same group of *Aspergillus terreus* according to morphological identification. *Aspergillus ochraceus* isolate 15 was separated from the rest of *Aspergillus ochraceus* isolates, i.e. 4, 12, and 16. Isolate 15 was clustered with *A. parasiticus* isolate 11 at 25% similarity but isolates 4 and 12 formed a separate cluster at 47% similarity. *Asperigillus parasiticus* isolates 19 and 20 formed one cluster at 50% similarity.

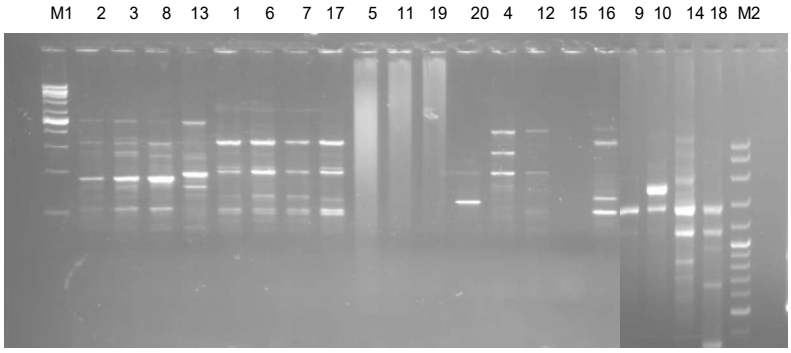


Figure 7. RAPD profile of 20 isolates of *Aspergillus* species with OPB 11 primer (M1=1 Kb ladder, M2=100 bp ladder).

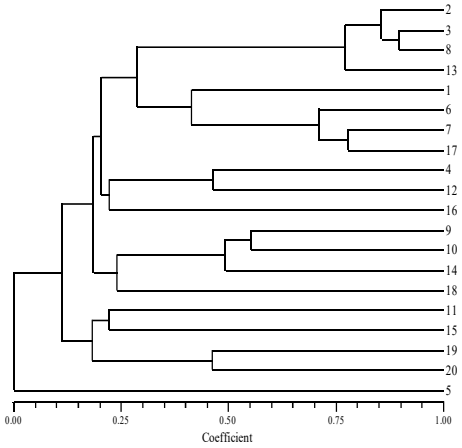


Figure 8. Dendrogram from RAPD analysis of *Aspergillus* isolates with combined primers.

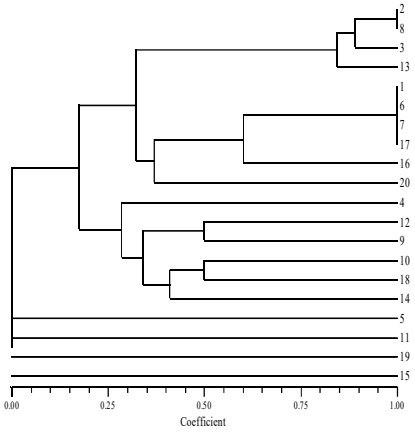


Figure 9. Dendrogram from RAPD analysis of *Aspergillus* species with primer OPB 11.

UPGMA cluster analysis with primers OPB11 and OPZ19 was also undertaken as the two primers showed a very distinct pattern differentiating clearly *Aspergillus* isolates. The obtained dendrogram (Fig. 9) revealed the lack of DNA amplification using OPB11 arbitrary 10 mer primer in some of *A. parasiticus* isolates (nos. 5, 11 and 19*)* and *A. ochraceus* isolates (no. 15). The analysis revealed two main clusters. In the first cluster, there are two sub clusters in which *Aspergillus flavus* isolates (nos. 1, 6, 7 and 17) showed 100% similarity. *Aspergillus niger* isolates 2 and 8 were identical, whereas isolates 3 and 13 formed one cluster at 80% similarity. *Aspergillus ochraceus* isolate 16 formed one cluster with *A. flavus* isolates at 60% similarity and isolate 20 of *A. parasiticus* also formed cluster with same species at 35% similarity. In the second sub cluster isolate 4 formed a separate cluster with 24% similarity to other isolates within sub cluster. *Aspergillus terreus* isolate 9 shared 50% similarity with *Aspergillus ochraceus* isolate 12. *Aspergillus terreus* isolate 10, 14 and 18 clustered together at 42% similarity.

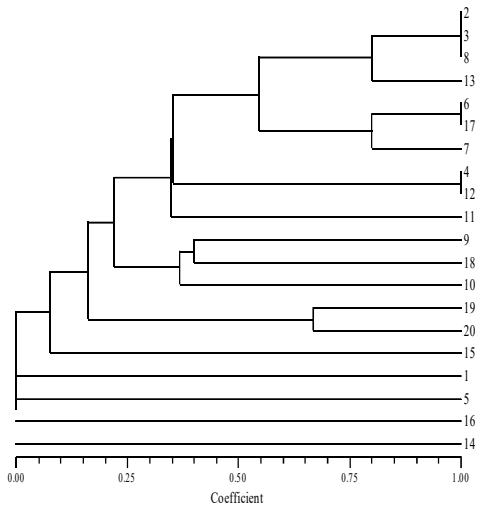


Figure 10. Dendrogram from RAPD analysis of *Aspergillus* species with primer OPZ19.

The RAPD analysis revealed that DNA was not amplified with primer OPZ19 in some *Aspergillus* species isolates (nos. 1, 5, 14 and 16) (Fig. 10). Isolates 2, 3, and 8 were identical but isolate 13 formed a distinct cluster with these isolates of same group at 80% similarity. Isolate 7 was distantly related and shared 80% similarity with isolates 6 and 17 but isolate 6 shared 100% similarity with isolate 17. There was 35% relatedness between isolates 11 and isolates 4 and 12, which shared 100% similarity among themselves. Isolate 9, 10 and 18 formed one cluster at 36.7% similarity. Isolates 19 and 20 were found related and shared 67.6% similarity.

CONCLUSION

The morphological and molecular studies revealed that various *Aspergillus* species are found in association with fruit and cereal crop species in the Delhi market. Randomly amplified polymorphic DNA (RAPD) fingerprints were used to analyze the genetic relationships among the isolates of *A. niger, A. flavus, A. parasiticus, A. terreus* and *A. ochraceus* which were identified into separate groups with the help of morphological characteristics. The study gave preliminary informative DNA-based markers for five species of *Aspergillus* identification

A good agreement was obtained between morphological and molecular characteristics. The investigation showed and proved that OPB11can be used as a specific primer for the molecular identification of the species *A. flavus*. as it gave monomorphic band for all the *A. flavus* isolates studied (Devi et al., 2013).

The phylogenetic analysis revealed that the *Aspergillus* species isolates collected from Delhi market are genetically diverse. These isolates of *Aspergillus* species are known to produce mycotoxins that are harmful to animal including human being. Therefore, attention should be given for designing management strategies to reduce the risk from mycotoxins.

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