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Diversity of *Aspergillus* species recovered from seeds of *Capsicum annuum* Linn. from Jammu

Shallu Samyal and Geeta Sumbali

Abstract: Mycological survey of red chilli seeds (19 samples) collected from Jammu division revealed an association of 11 *Aspergillus* species. Among the various *Aspergillus* species recovered, percent frequency of *A. niger* was highest (68.0%) followed in decreasing order by *A. flavus* (58.0%). Few other potentially toxigenic species of *Aspergillus* viz., *A. sydowii*, *A. versicolor* and *A. ochraceus* were detected from 16.0% chilli seed samples. Other *Aspergillus* species like *A. flavipes*, *A. ficuum*, *A. nidulans*, *A. parasiticus*, *A. terreus* and *A. terricola* var. *americana* were of rare occurrence having a percentage frequency of 5.0%. Contamination of seeds by *Aspergillus* species adversely affects the quality of seeds as many of them produce mycotoxins causing health hazard in human beings.

Key words: *Aspergillus* species; *Capsicum annuum*; health hazard; mycotoxins.

1. Introduction

Chilli seeds host many storage fungi during post-harvest period that reduce the seed quality. The species of storage fungi predominately belong to two genera viz., *Aspergillus* and *Penicillium*. Among these, aspergilli are more prevalent because even minor increase of atmospheric humidity leads to their growth (Panasenko, 1967). Fungi associated with stored seeds are chiefly responsible for their deterioration and reduction in germination potential (Pant 2011). In addition, quality of seeds and fruits of *Capsicum* is reduced due to infestation o f fungi that produce mycotoxins causing health hazard in human beings (Hiscocks, 1965). In view of the visual changes observed in the colour of capsicum seeds, an attempt was made to investigate them for the diversity of various *Aspergillus* species.

2. Materials and methods

2.1 *Mycological studies*

Aspergillus species associated with the chilli seeds were assessed by adding 5g of ground seed sample to 45 ml of sterile water in 250 ml Erlenmeyer flask. The sample was homogenized thoroughly on an electric shaker at a constant speed for 15 minutes and then tenfold serial dilutions were prepared. One ml portion of suitable dilution was used to inoculate sterilized petridishes. For the recovery of maximum number of *Aspergillus* species, three different media – Czapek Dox agar (CDA), dichloran 18% glycerol agar (DG_{18}) and malt salt agar (MSA) were used, and for each medium five replicates were maintained. The medium was poured by making gentle rotational movement of petriplates so as to ensure uniform spreading of the sample. Petriplates thus prepared were incubated at 28 $\pm$ 2ºC for 7 days. Finally, the aspergilli colonies were counted and the results were expressed as average colony forming units in thousands per gram of sample (10^4 cfu/g) using the following formula (Parikh and Shah, 2006).

$$\text{cfu/g} = \frac{\text{a x d}}{\text{s}}$$

a = average number of colonies on the petriplate

d = dilution factor

s = dry weight of sample taken

Percentage frequency of occurrence (%) of each *Aspergillus* species was calculated by using the following formula:

$$\text{Frequency (\%)} = \frac{\text{No. of samples from which } \textit{Aspergillus} \text{ was recorded}}{\text{Total number of samples tested}} \text{x100}$$

2.2 Purification, maintenance and identification of Aspergillus species recovered from the capsicum seeds

Aspergillus species recovered from the samples were purified by streaking and maintained on sterilized potato dextrose agar (PDA) medium slants at 8-10ºC. Subsequent sub-culturing was done after every six months to maintain the viability and purity of cultures. Each isolate was cultured and maintained in triplicate. Identification of the recovered *Aspergillus* species was

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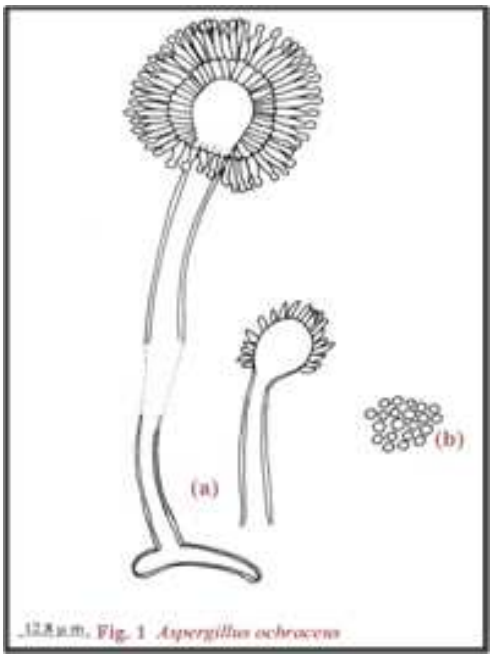
done by studying their cultural and morphological characters and following the key given by Raper and Fennel (1965). Drawings of the recovered aspergilli were made with Erma make camera lucida and micro measurements were recorded with the help of Erma make ocular micrometer that was calibrated with stage micrometer at different lens combitions (100x, 400x and 1000x).

3. Results and Discussion

During the present investigation, 11 species of aspergilli were detected to be associated with the seeds of *Capsicum annuum* their camera lucida drawings and brief description are tabulated below.

3.1 *Aspergillus ochraceus* Wilhelm

Colonies on MEA growing rapidly and attaining a diameter of 35 – 40 mm in 7 days at 28 ± 2ºC, plane, sometimes more or less zonate with vegetative mycelium submerged; conidial heads yellow, globose when young, later splitting into two to more compact columns.

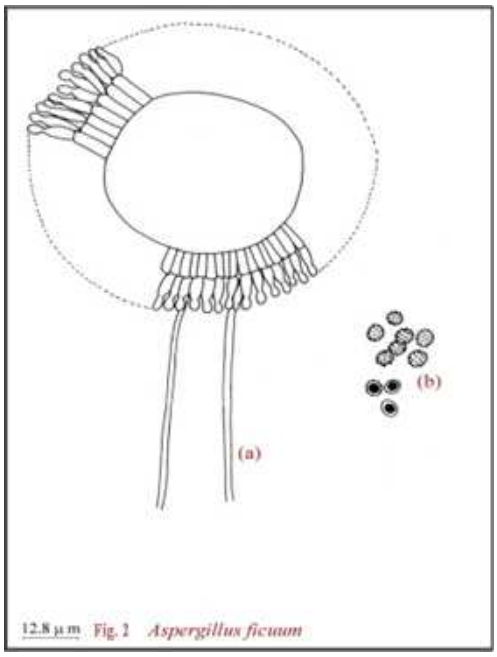


Conidiophores long, pale, yellow, smooth; vesicles globose, hyaline, 24.0 – 43.6 µm in diameter; sterigmata biseriate; metulae 9.4 – 16.0 x 4.0 – 5.3 µm; phialides ampulliform, measuring 7.0 – 9.6 x 2.0 – 3.0 (Fig., 1a); conidia globose to subglobose, mostly 2.5 – 3.2 µm in diameter, smooth (Fig., 1b).

3.2 *Aspergillus ficuum* (Reich.) Hennings

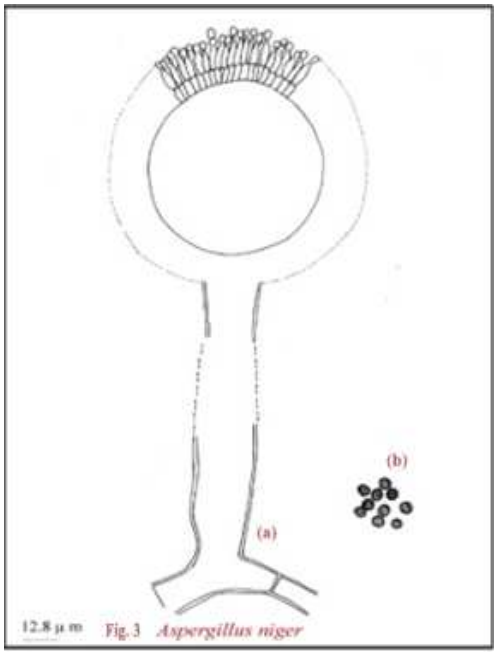
Colonies on MEA growing rapidly, attaining a diameter of 40 – 50 mm in 5 days, basal mycelium hyaline, inconspicuous; conidial heads carbon black, abundant and crowded, but thinning at the margins; reverse pale to faintly yellow.

Conidiophores erect, pigmented brown, measuring 500–1500 mm in length and 15 – 20 µm in width; vesicles globose, pigmented, 25 – 70 µm in diameter, fertile over the entire surface; sterigmata in two series; phialides 7 – 10 x 3.0 – 3.5 µm, often crowded (Fig., 2a); conidia globose, 3.5 – 4.0 µm in diameter, with coarse striations (Fig., 2b).



3.3 *Aspergillus niger* Van Tieghem

Colonies on MEA fast growing, attaining a diameter of 40 – 50 mm in 7 days at 28 ± 2ºC, plane or slightly floccose, heavily sporulating throughout. Conidial heads black, radiating and tend to split into loose columns with age.



Conidiophores borne from subsurface hyphae, smooth, hyaline to pale brown, particularly in the upper half, commonly 1.5 – 2.5 mm in length; vesicles globose to subglobose, 40.0 – 68.8 µm in diameter, bearing closely packed metulae and phialides over the

whole surface; phialides 7.0 – 10.0 x 2.8 – 3.3 µm (Fig., 3a); conidia spherical, 4.0 – 5.0 µm in diameter, brown, ornamented with irregular spines and ridges (Fig., 3b).

3.4 *Aspergillus flavus* Link

Colonies on MEA rapidly growing, attaining a diameter of 35 – 45mm in 7 days at 25 ± 2ºC; basal mycelium thin and mostly submerged, commonly plane but occasionally radially furrowed or wrinkled; conidial head light yellow green, later becoming dark yellow green; sclerotia produced in many strains, sometimes dominating the colony appearance.

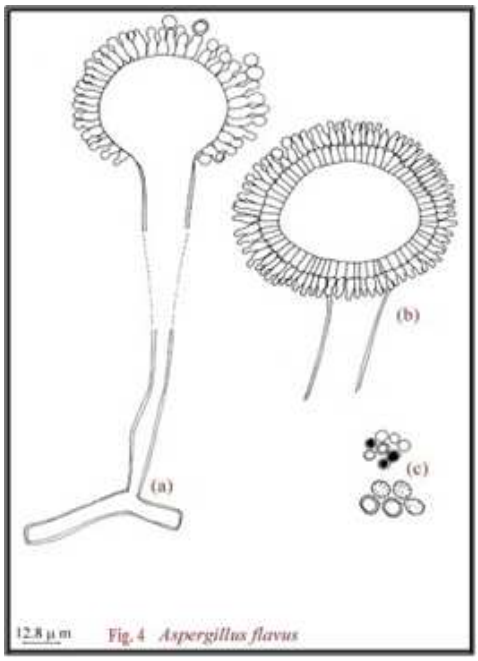


Fig. 4 *Aspergillus flavus*

Conidiophores coarsely rough, upto 1mm long; heads varying in size, loosely radiate or splitting; vesicles subglobose to globose, varying in diameter from 9.6 – 62.4 µm; sterigmata biseriate but having some heads with phialides borne directly on the vesicle (Fig., 4g and h); phialides 7 – 10 x 2 – 2.5µm; conidia usually globose to subglobose, conspicuously roughened, 3.2 – 6.0 µm in diameter (Fig., 4 c).

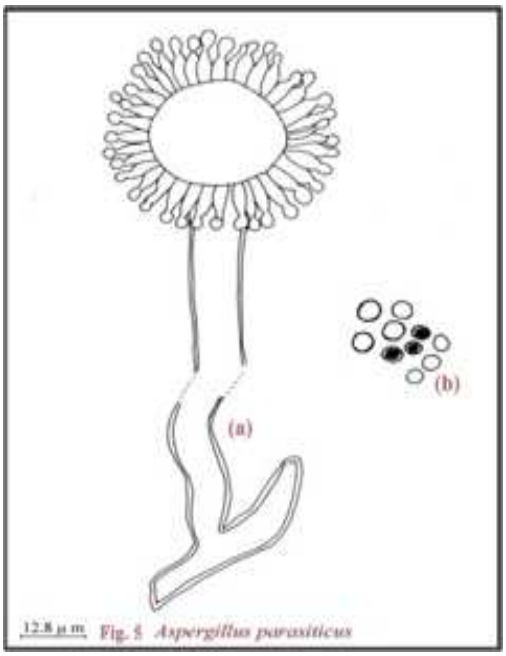


Fig. 5 *Aspergillus parasiticus*

3.5 *Aspergillus parasiticus* Speare

Colonies on MEA spreading rapidly, attaining a diameter of 30-35 mm in 5 days at 28 ± 2ºC; basal mycelium submerged, heavily sporulating, parrot green, plane to somewhat flocculent; reverse pale.

Conidiophores 200 – 650 µm in length, hyaline; vesicles 20 – 35 µm in diameter; sterigmata uniseriate, 6.4 – 8.8 x 2.8 – 4.0 µm (Fig., 5a); conidia globose, 3.2 – 4.8 µm in diameter (Fig., 5b).

3.6 *Aspergillus terricola* var. *americana* Marchal.

Colonies on CYA 30 – 35 mm in 7 days at 28 ± 2ºC, somewhat floccose in centre, changing from ochraceus yellow to yellowish brown; conidial heads loosely radiate; reverse colourless. Colonies on MEA dense, centrally raised, colouration same as that on CYA.

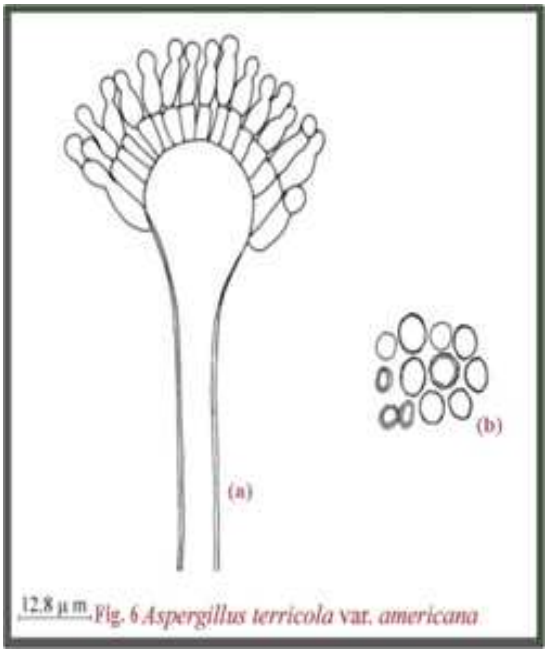


Fig. 6 *Aspergillus terricola* var. *americana*

Conidiophores 240 – 450 µm long, hyaline; vesicles subglobose, 12.8 – 30.0 µm in diameter, fertile over the upper two third; sterigmata mostly in one series, but with both uniseriate and biseriate structures in the same head also present; metulae 4.8 – 6.8 x 3.2 µm; phialides ampulliform, hyaline, measuring 6.4 – 8.0 x 2.5 – 3.0 µm (Fig., 6a); conidia globose, 3.2 – 4.8 µm in diameter, rugulose, brownish yellow (Fig., 6b).

3.7 *Aspergillus sydowii* (Bain. and Sart.) Thom & Church

Colonies on MEA 25 – 30 mm in 7 days at 28 ± 2ºC, plane; conidial heads blue- green, radiate to nearly globose; reverse reddish maroon.

Conidiophores hyaline, 272-400 µm in length, smooth; vesicles club-shaped, fertile over almost the entire surface, 6.4 – 12.8 µm in diameter. Sterigmata in two series; metulae 4.8 – 6.4 x 2.8 – 3.2 µm;

phialides 6.4 – 9.6 x 1.9 – 2.4 µm; fragmentary structures abundant (Fig., 7a and b). Conidia globose to subglobose, spinulose, 2.5 – 3.5 µm in diameter (Fig., 7c).

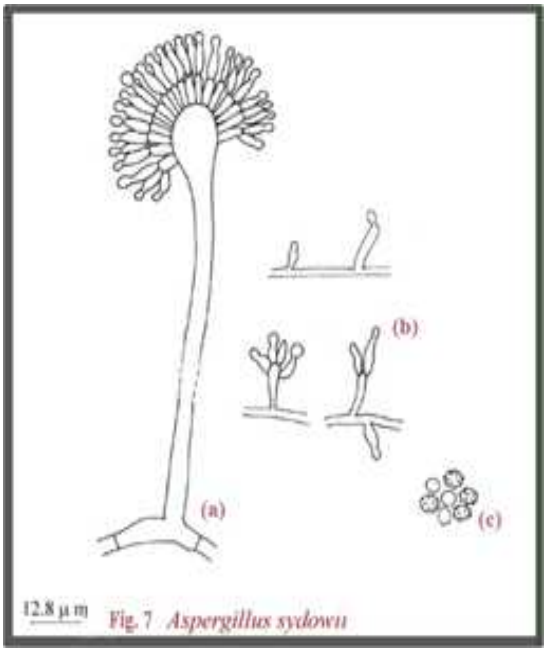


Fig. 7 *Aspergillus sydowii*

3.8 *Aspergillus versicolor* (Vuill.) Tiraboschi

Colonies on MEA growing rapidly, 25 – 35 mm in 7 days at 28 ± 2 ºC with considerable range in colour from pale green, dry green to buff, heavily sporulating, occasionally in flesh to pink shades; exudates absent to abundant, ranging from clear to dark wine shades, somewhat flocculent; reverse in yellow orange to purple red shades.

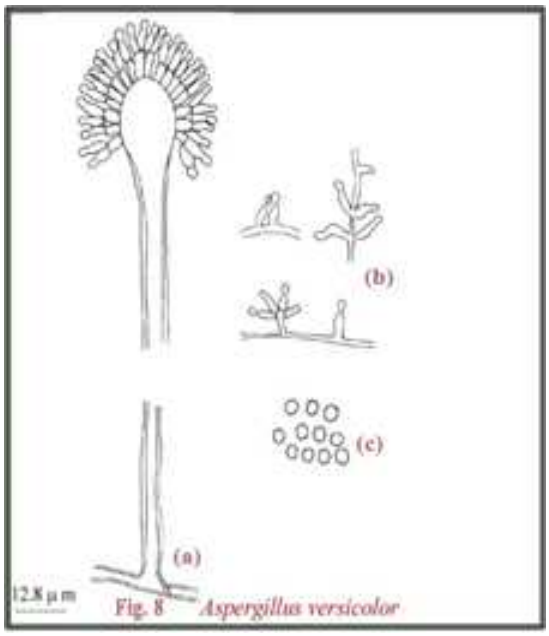


Fig. 8 *Aspergillus versicolor*

Conidiophores hyaline to yellowish, heavy-walled; vesicles hemispherical to elongated, measuring 9.6 – 17.6 µm in width; fragmentary heads abundant (Fig., 8a); sterigmata in two series, covering only upper surface of vesicles; metulae 6.4 – 8.0 x 3.2 µm; phialides 5.0 – 6.4 x 2.0 – 2.4 (Fig., 8b); conidia globose, delicately roughened, 2.5 – 3.3 µm in diameter (Fig., 8c).

3.9 *Aspergillus nidulans* (Eidam) Wint.

Colonies on MEA growing rapidly and attaining a diameter of 45 – 50 mm in 7 days at 28 ± 2 ºC, plane, deep yellow green; reverse purplish. Conidial heads short, columnar.

Conidiophores smooth-walled, in shades of cinnamon brown, ranging from 60 – 120 µm in length, 2.5 – 3.0 µm in width near the foot, increasing to 3.5 – 5.0 µm below the hemispherical vesicles. The vesicles measure 8.8 – 10.4 µm in diameter; sterigmata biseriate; metulae 4.8 – 6.4 x 2.0 – 2.4 µm; phialides 4.8 – 6.4 x 2.0 – 2.4 µm (Fig., 11a); conidia globose, rugulose, 3.2 – 3.3 µm in diameter (Fig., 9b); hulle cells present (Fig., 9c).

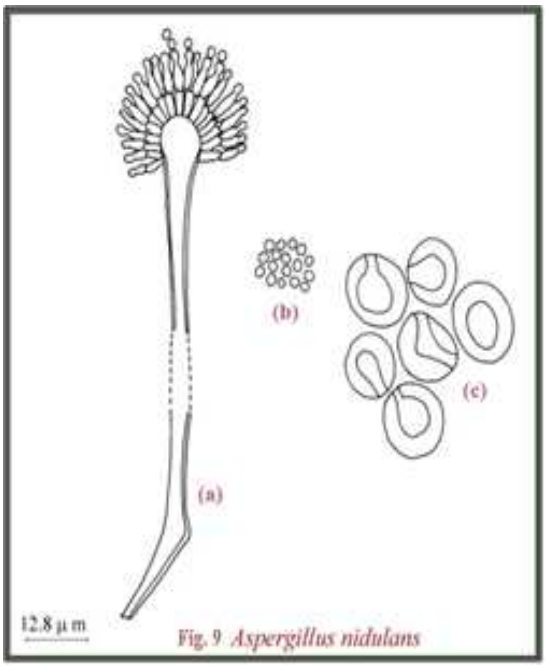


Fig. 9 *Aspergillus nidulans*

3.10 *Aspergillus flavipes* (Bain. and Sart.) Thom and Church

Colonies on MEA attaining a diameter of 30 – 40 mm in 7 days at 28 ± 2 ºC, plane, velvety, zonate, sporulating abundantly in pale to dull pinkish buff shades; exudate lacking; reverse in brown shades. Conidial heads loosely columnar.

Conidiophores comparatively longer at the margins of older colonies, commonly 2.0 – 2.5 mm in length; vesicles subglobose to vertically elongate, 17.6 – 22.4 µm in diameter; sterigmata in two series, borne on only upper portion of vesicle in small heads, crowded and covering the vesicle in large heads; primaries about 5.1 – 7.2 x 2.4 – 4.8 µm; secondaries 5.0 – 7.0 x 2.0 – 2.8 µm (Fig., 10a and b); conidia globose to subglobose, smooth, 2.0 – 3.0 µm in diameter (Fig., 10c).

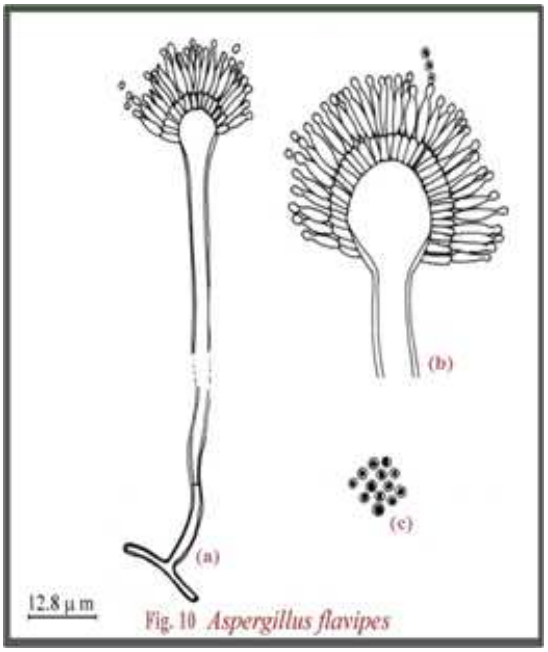


Fig. 10 *Aspergillus flavipes*

3.11 *Aspergillus terreus* Thom.

Colonies on MEA growing rapidly, attaining a diameter of 40 – 50 mm in 7 days at 28 ± 2ºC, thin, plane, producing abundant conidial heads in cinnamon to orange brown shades; reverse creamy yellow.

Conidial heads long, columnar, compact, uniform diameter throughout; vesicles hemispherical, dome-like, commonly 11.0 – 17.6 µm in diameter; sterigmata in two series; primaries crowded, parallel, 4.8 – 6.4 x 2.0 – 2.4 µm; secondaries closely packed, 5.6 – 7.5 x 1.6 – 2.0 µm (Fig., 11a); conidia globose to slightly elliptical, smooth, commonly 1.8 – 3.0 µm in diameter (Fig., 11b).

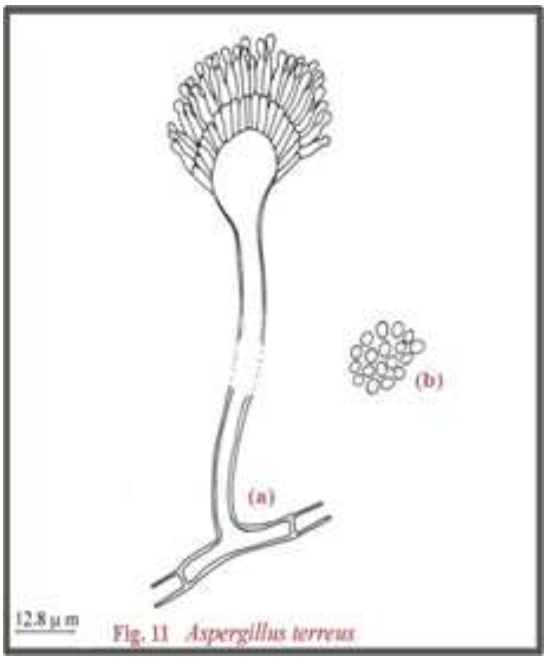


Fig. 11 *Aspergillus terreus*

Data presented in table 1 shows that among the *Aspergillus* species that were recovered from the seeds of *Capsicum annuum* the seeds of dried red chillies, frequency percent of *A. niger* was the highest (68.0 percent) followed in decreasing order by *A. flavus* (58.0 percent). These results are consistent with the findings of Sharfun-Nahar *et al*. (2004) who also found *A. flavus* and *A. niger* showing maximum occurrence percentage on the seeds of *Capsicum annuum* imported from India. Recently, Kumari (2012) while investigating the seed borne mycoflora of chilli seeds collected from different locations of Gujarat also noticed that *A. niger* was dominating followed in decreasing order by *A. flavus*. During the present study, few other potentially toxigenic species of *Aspergillus* viz., *A. sydowii*, *A. versicolor* and *A. ochraceus* were detected from 16.0 percent chilli seed samples. However, *Aspergillus* species like *A. flavipes, A. ficuum, A. nidulans, A. parasiticus, A. terreus* and *A. terricola* var. *americana* were of rare occurrence having a percentage frequency of 5.0 percent. Hedawoo and Chakranarayan (2011), while studying the fungal diversity of some other Indian seed spices also found *Aspergillus flavus, A. fumigatus, A. niger* and *A. ochraceus* common on all of them with *A. niger* showing highest percentage of incidence.

During the present investigation, spore count of various *Aspergillu*s species on chilli seeds was found to be quite high. It was highest for *A. niger* (9.4×10^2 – 1.1×10^3 cfu/g), followed in decreasing order by that of *A. flavus* (5.0×10^2 – 8.4×10^2 cfu/g). Earlier, Kiran *et al*. (2005) also reported *Aspergillus* as the predominant fungus on the surface sterilized and unsterilized chilli seeds from Andhra Pradesh. Similarly, Kulkarni *et al*. (2005) recovered *A. niger* as the dominant fungal species from chilli seeds of Ahmednagar.

During the present investigation, other toxigenic species of *Aspergillus* viz., *A. sydowii*, *A. versicolor* and *A. ochraceus* were detected from chilli seed samples with colony count ranging from 7.3×10^1 – 1.4×10^2, 1.0×10^2 – 1.5×10^2 and 1.1×10^2 – 2.3×10^2 cfu/g respectively. Six other *Aspergillus* species (*A. flavipes*, *A. ficuum*, *A. nidulans*, *A. parasiticus*, *A. terricola* var. *americana* and *A. terreus* were detected to show rare occurrence with a total colony count ranging from 2.1×10^1 -7.3×10^1 cfu/g. Colonization of the seeds by aspergilli was usually observed to change the normal ivory white colour of the seeds to yellow, olive green, grey or black. Usually they become yellow in pods containing *A. ochraceus*, black in pods containing *A. niger* and olive green in pods with *A. flavus*. The changes in the colour of chilli seeds were not merely due to dusting with conidia, but as a result of colonization followed by profuse conidiophore and conidia production. A seed- borne pathogen present externally or internally or associated with the seed as a contaminant may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage or development of disease at later stages of plant growth (Khanzada *et al*., 2002). Basak *et al*.

(1996) suggested that many of the fungal species associated with chilli seeds could even cause fruit rot disease of chilli. These fungi also produce mycotoxins causing health hazard in human beings (Samyal, 2013). Therefore, management of aspergilli contamination during production, harvesting and post-harvest processing is very necessary for which more studies are required.

Table 1. Frequency percent and total colony count of *Aspergillus* species recovered from the seeds of dried red chillies on different growth media.

Fungal Species recovered	% Freq.	cfu/g		
		CDA	DG-18	MSA
Aspergillus flavipes	5.0	2.1×10^1	-	-
A. ficuum	5.0	-	5.2×10^1	-
A. flavus	58.0	8.4×10^2	6.4×10^2	5.0×10^2
A. nidulans	5.0	-	3.1×10^1	-
A. niger	68.0	9.2×10^2	1.1×10^3	1.1×10^3
A. ochraceus	16.0	1.1×10^2	2.3×10^2	1.3×10^2
A. parasiticus	5.0	4.2×10^1	-	-
A. sydowii	16.0	9.4×10^1	1.4×10^2	7.3×10^1
A. terreus	5.0	7.3×10^1	-	-
A. terricola var. *americana*	5.0	5.2×10^1	-	-
A. versicolour	16.0	1.0×10^2	1.5×10^2	1.0×10^2

- Not detected

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