Diversity of Guanophilic Fungi of Frugivorous Bats

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Introduction

Bat guano supports a great diversity of organisms including arthropods that live on guano (Ferreira and Martins, 1998) and energy flow in these tropic cascades based on guano production by roosting bats (Hairston and Hairston, 1993). Interestingly, the diversity of organisms living on or in guano piles differs depending on the diet of the bat producing the guano. Guano from sangivorous bats is typically inhabited by fly larvae springtails, and beetles. Guano of insectivorous bats inhabited by mites, pseudocorpions, beetles, thrips, moths and flies lastly guano of frugivorous bats is inhabited by spiders, mites, isopods, millipedes, centipedes, springtails, bark lice, true bugs, and beetles (Ferreira and Martins, 1998). Although, several studies have compared the impact of guano from different bat species on cave ecology (Trajano, 1996; Ferreora and Martins, 1998; Shahack-Gross *et al.*, 2004), the composition of guano from bats consuming different diets has received little attention. Fungi are distributed worldwide, with particular species being endemic in particular regions. The species are grouped by natural environment as being primarily associated with humans (Anthrophilic), other animals (zoophilic), or soil and guanophilic (Brandt and Warnock, 2003).

Bat guano is rich substrates for fungi in the cave environment along with dung, plant debris, carcasses and other organic debris. Poulson (1972) reported that bat guano is simple in structure but just enough to constitute a complete ecosystem. The fungi present in guano commonly serve as saprotrophs andor pathogens as transient chemo-heterotrophic microorganisms (Northup *et al.*, 1997). Therefore, the present study was aimed to investigate the diversity of guanophilic fungi in the guano of fruit eating bats.

Materials and Methods

Guano samples of *Rousettus leschenaulti* were collected from the historical monuments of Ayodhya, Faizabad(26°45'58"N 82°08'40" E), Bara Imambara, Lucknow (26°86'85" N, 80°91'27" E) and Chunar Fort, Mirzapur(25°07'15.02"N 82°52'34.77" E) while the samples of *Cynopterus sphinxcollected* from Babasaheb Bhimrao Ambedkar University campus, Lucknow (26°76'57"N, 80°92'09" E) and Sidharth Nagar (27°27'16" N, 82°82'10" E). The samples of *Pteropus giganteus* were collected from Mohanlalganj (26°40'57"N, 80°59'1.49"E).The guano samples were collected aseptically using spatula and forceps, kept in sample vials and stored at -20°C for further analysis.

Isolation of fungus

The guano samples were diluted serially by following Aneja (2003). The diluted sample was inoculated in sterile petri dishes containing potato dextrose agar medium supplemented with chlorotetracycline. The plates were incubated at 28°C for 8days. Thereafter, the properly grown fungi were used for subculture in Czapck yeast agar plates and incubated at 25°C for 3-7 days.

Microscopic analysis

A small portionof mycelium and conidiophores were extirpatedfrom young colony, placed on a microscopic slide and gently spread it. A drop of lactophenol cotton blue was taken on a glass slide, observed under Light Microscope (OLYMPUS CX41) and photographs were taken at different magnifications.The fungal species were identified based on morphological features and by following Thom (1945) and Raper and Thom (1949)

Results and Discussion

A total of 13 fungal isolates were obtained from

Figure 01. Colony morphology of *Aspergillus niger* (A), *A. versicolor* (B), *Aspergillus* sp. (C), *Aspergillus* sp.(D), *Aspergillus* sp. (E), *Aspergillus* sp. (F), *Aspergillus* sp.(G), *Penicilliumcitrinum*(H), *Penicillum* sp.(I), *Penicillum* sp. (J), *Fusarium* sp. (K), *Mucor* sp.(L), *Alternaria* sp. (M).

the guano samples of three fruit eating bats. The guanophilic fungi such as Aspergillusniger, *A.versicolar* and *Penicilliumcitrinum*were identified to species level (Fig. 1). In addition four fungal isolates belong to genera Aspergillus, two isolates belong to Penicillium and each one isolate of genera Fusarium, Mucorand Alternaria were observed (Fig. 1).

The colony of *A. niger* attained an average diameter of 80 mm (Fig.1A), the conidial head wascarbon black, large and globose (Fig. 2A).The septate hyphae of *A. niger* were translucent. The colony of *A. versicolar* attained 17.52 mm at maturity, the hyphaeboar chains of rough conidia on

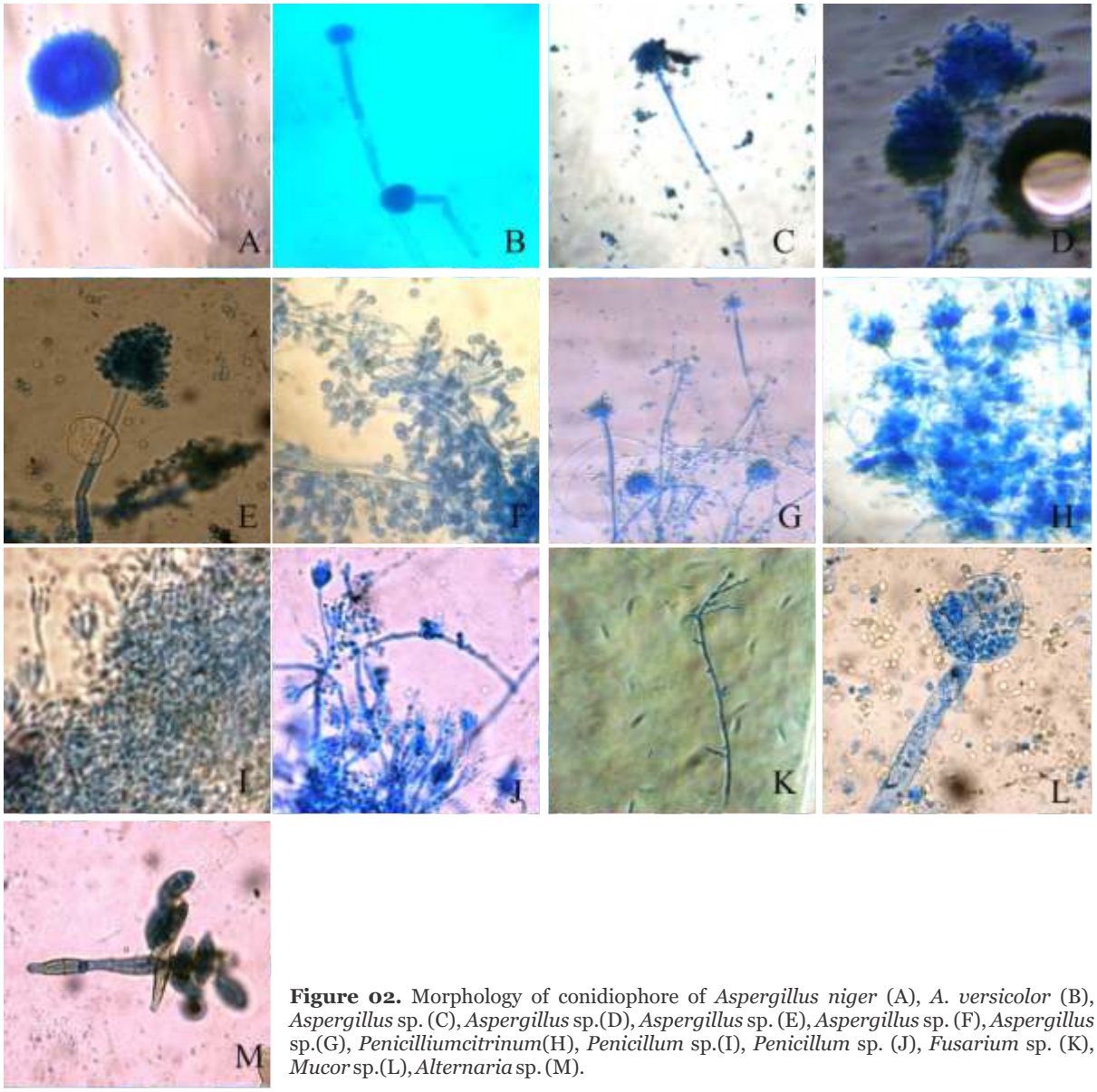

Figure 02. Morphology of conidiophore of *Aspergillus niger* (A), *A. versicolor* (B), *Aspergillus* sp. (C), *Aspergillus* sp.(D), *Aspergillus* sp. (E), *Aspergillus* sp. (F), *Aspergillus* sp.(G), *Penicilliumcitrinum*(H), *Penicillum* sp.(I), *Penicillum* sp. (J), *Fusarium* sp. (K), *Mucor* sp.(L), *Alternaria* sp. (M).

terminal ends (Fig. 2B). In addition,five isolates belong to genus Aspergillus were observed in the guano sample(Fig. 1C – 1G,Fig. 2C – 2G).

The colony of *Penicillium citrinum* was cottony, green with white margin, rounded and attained 16.04 mm (Fig. 1H). The conidiophore had alarge number of whip-like conidial chains withspherical spores (Fig. 2H). In addition, two isolatesof *Penicillium* were observed in the guanosamplesof fruit bats (Fig. 1I – 1J). The colony of *Fusarium* sp. was pale in color. The colony grown very fast and attained 90 mm diameter at maturity(Fig. 1K). The conidia are 1 to 2-celled, hyaline, fusiform to ovoid, curved (Fig. 2K). The colony of *Mucor* sp. was grey , attained 89 mm diameter at maturity (Fig. 1L). *Mucor* spores were simple and form apical, globular sporangia that were supported and elevated by column-shaped columella (Fig. 2L). The colony of *Alternaria* sp. was pale, attained 30 mmdiameter at maturity (Fig. 1L).

The results of present study revealed the existence of fungi in the frugivorous bat guano.

Further, the bat guano offers a suitable substratum and nutrients for the growth of fungus. Otomycosis (fungal ear infections), which causes pain, temporary hearing loss and damage to the ear canal and tympanic membrane caused by *A. niger*. *Aspergillus nigersecretes* toxic secondary metabolite which causes several ailments on almost all organs in human (Durakovic et al., 1989; Rai and Mehrotra, 2005). *Aspergillus versicolor* was known to cause severe lung problems (Aspergillosis) to human if inhaled in sufficient amount. *Fusarium* is a common soil fungus on a wide range of plants. Fungus belong to this genera causes hemorrhagic syndrome in humans (alimentary toxic aleukia) and characterized by nausea, vomiting, diarrhea, dermatitis and extensive internal bleeding.

Mucor sp. grow on living and dead plants. Since the frugivorous bats rely on various plant source for survival, accidentally they might have consumed the spores of Mucor sp. The mold of *Mucor* grown and spread quickly compared to other fungi. *Alternaria* is ubiquitous in the environment and it is one of the fungi which humans interact with most frequently, on a variety of levels. *Alternaria* species are a leading cause of crop blight, and they also cause allergies and infections in some people and animals.

Conclusion

The frugivorous bats such as *Pteropus giganteus, Rousettus leschenaulti* and *Cynopterus sphinx* are mainly rely on fruits, nectar, leaves and other plant resources. The guano of frugivorous bats harboured 13 fungal isolates such as *Aspergillus niger, A. versicolar* and *Penicillium citrinum,* four isolates belong to genera Aspergillus, two isolate belong to genus *Penicillium* and each one isolate belong to genera *Fusarium, Mucor* and *Alternaria*. Few species of fungi isolated from the guano of fruit batswere pathogenic to human and plants.The occurrence of plant fungal pathogen in the guano of fruit bats reveal that the frugivorous bats relied on the infected plant parts as source of food.

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