ISOLATION AND CHARACTERIZATION OF GUANOPHILIC FUNGI OF THE BATS OF UTTAR PRADESH

THESIS

SUBMITTED IN FULFILMENT FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

APPLIED ANIMAL SCIENCES

Under the supervision of
Dr. V. ELANGOVAN
Associate Professor

Submitted By
PAWAN KUMAR MISRA
M.Sc.

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

प्रज्ञा शील करुणा
ESTABLISHED 1996

DEPARTMENT OF APPLIED ANIMAL SCIENCES
SCHOOL FOR BIOSCIENCES AND BIOTECHNOLOGY
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226 025 (U.P.), INDIA

Enrolment No. 214/11

Year 2016

Dedicated

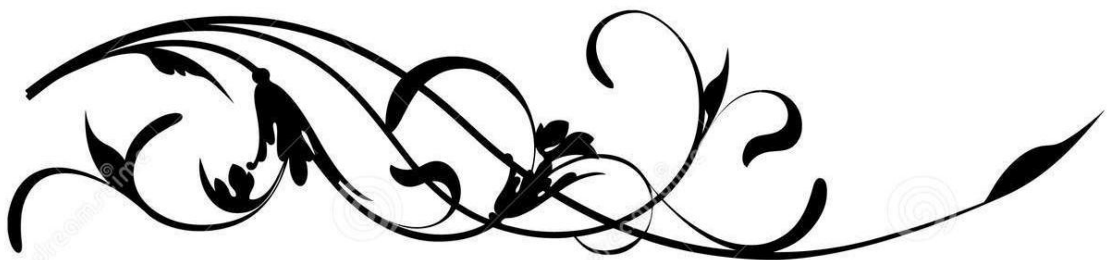
to my inspiring parents and brothers,

for being the

pillows, role models, catapults,

cheerleading squad and sounding boards

I have needed.



DECELERATION

I hereby declare that the thesis entitled "**ISOLATION AND CHARACTERIZATION OF GUANOPHILIC FUNGI OF THE BATS OF UTTAR PRADESH**" submitted by me for the degree of **Doctor of Philosophy** in **Applied Animal Science** is the result of my original work carried out under the guidance of **Dr. V. Elangovan,** Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow and it has not been submitted for the award of any degree, diploma, associateship of any University or Institution.

Place: Lucknow Signature of candidate

Date:

(Pawan Kumar Misra)

CERTIFICATE

This is certify that the thesis titled "**Isolation and characterization of guanophilic fungi of the bats of Uttar Pradesh**" submitted by **Pawan Kumar Misra** is original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or other university.

The thesis is submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) regulation – 1999 as amended in 2008/2010 and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: **Supervisor**

Dr. V. Elangovan

(Associate Professor)

Head of Department

Dr. V. Elangovan

(Associate Professor)

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor Dr. Vadamalai Elangovan (Associate Professor) of the Department of Applied Animal Sciences, School of Biosciences and Biotechnology at Babasaheb Bhimrao Ambedkar University, Lucknow. The door of Elangovan sir office was always open whenever I ran into a trouble spot or had a question about my research or writing. He consistently allowed this thesis to be my own work, but steered me in the right direction whenever he thought I needed.

I would also like to thank the experts Dr. Manoj Kumar Upadhay (Scientist C), Biotech Park, Lucknow providing lab equipment and continuous guidance in my experiment. Without their passionate participation and input, the validation survey could not have been successfully conducted.

I would also like to acknowledge all faculty members of Department of Applied Animal Sciences, School of Biosciences and Biotechnology at Babasaheb Bhimrao Ambedkar University, Lucknow as the second reader of this thesis, and I am gratefully indebted to their very valuable comments on this thesis.

I sincerely thank Dr. Yuvana Satya Priya, Assistant Professor of Remedial Coaching Academy, Babasaheb Bhimrao Ambedkar University, Lucknow, who was deeply involved in the validation survey for this research project and her valuable guidance. She definitely provided me with the tools that I needed to choose the right direction and successfully completed my thesis writing.

I thank my senior Dr. Mukesh Kumar and Dr. Harendra Kumar and my fellow labmates Mr. Deep Narayan Prasad, Mr. Ram Kumar, Mr. Yogesh Kushwah and Mr. Rajan Verma for the stimulating discussions, for the sleepless nights we were working together before deadline, and for all the fun we have had in the last four years. Also I

thank my friends in University of Lucknow, Lucknow: Miss Neelam Kumari Gautam and from Central Drug Research Institute: Pawan Kumar Yadav for enlightening me the first glance of research.

I would also like to thanks Miss Aradhana Singh and Miss Divya Sharma, M. Sc. students, who helped me in lab work during my research work.

I would also like to thank my parents for their wise counsel and sympathetic ear. He was always there for me. Finally, there are my friends.

I thank the department staff member of Department of Applied Animal Sciences of support and favoured.

Last but not the least, I would like to thank to University Grant Commission under the Department of Empowerment of person with Disabilities (Divyangjan), Ministry of social Justice and empowerment, financial support.

Pawan Kumar Misra

List of Contents

	Page No.
Deceleration	i
Certificate	ii
Acknowledgement	iii - iv
List of Tables	v - vi
List of Figures	vii - xv
Abbreviations	xvi
General Introduction	**1- 3**
Review of Literature	**4 - 18**
Chapter 01: Roosting Ecology of Bats	**19 - 32**
Introduction	
Materials and Methods	
Results	
Discussion	
Chapter 02: Isolation and identification of guanophilic fungi	**33 - 74**
Introduction	
Materials and Methods	
Results	
Discussion	
Chapter 03: Characterization of guanophilic fungi using Fourier Transformed Infrared Spectroscopy (FTIR)	**75 - 99**
Introduction	
Materials and Methods	

Results

Discussion

Chapter 04: Diet analysis and diet composition of bat guano **100 - 123**

Introduction

Materials and Methods

Results

Discussion

Summary **124 - 126**

Conclusion **127 - 128**

References **129 - 152**

LIST OF TABLES

		Page No.
Table 1.1	Roost sites, roost characteristics and microclimate of bats.	28 – 30
Table 2.1	Colony characteristics of guanophilic fungi isolated from the guano of ten bats species.	50 – 55
Table 2.2	Morphological identification, Gen Bank accession numbers and most resemblance BLAST search for sequences of the fungal isolates from the guano of six insectivores bat.	56 – 57
Table 2.3	Morphological identification, Gen Bank accession numbers and most resemblance BLAST search for sequences of the fungal isolates from the guano of two frugivorous bats.	58 – 59
Table 3.1	Shows the wavenumbers, oscillations of functional groups (*) and guanophilic fungal isolates.	85 – 88
Table 4.1	Occurrence of insect orders from the guano of insectivorous bats.	112
Table 4.2	Percent elemental share in the guano of different species of insectivorous bats. Values are given as mean ± SD. Sample size is given in parenthesis.	113

LIST OF FIGURES

		Page No.
Figure 1.1	Different species of bats observed in the study area: *Rousettus leschenaulti* (A), *Cynopterus sphinx* (B), *Rhinopoma hardwickii* (C), *R. microphyllum* (D), *Scotophillus heathii* (E), *Scotophillus kuhlii* (F), *Pipistrellus coromandra* (G), *Taphozous nudiventris* (H), *Megaderma lyra* (I) and *Hipposideros fulvus* (J).	31
Figure 1.2	The roosts of different bat species: *Rousettus leschenaulti* (A), *Cynopterus sphinx* (B), *Rhinopoma hardwickii* (C), *Rhinopoma microphyllum* (D), *Scotophilus heathii* (E), *Scotophilus kuhlii* (F), *Taphozous nudiventris* (G), *Megaderma lyra* (H) and *Hipposideros fulvus* (J).	32
Figure 2.1	Amplification products obtained from gDNA isolated from guano of bats amplified with ITS4 and ITS5 primers. Lane L: ladder, lane 1: *Mucor* sp., lane: 2 – 21 *Aspergillus* sp., lane 22 – 26 *Penicellium* sp., lane 27 – 28 *Cladosporium* sp., lane 29 – 30: *Alternaria* sp., lane 31: *Sarcocladium* sp., lane 32: *Pericornia* sp., lane 33: *Trichocomaceae* sp.	60
Figure 2.2	Phylogenetic analysis of fungi isolated from the guano of insecivores bat was performed based on ITS4 and ITS5 gene data using neighbour joining (NJ) with bootstrap value 1000 replications. Mucor indicus strain CCUG22424SSH were included as outgroups in ITS phylogeny.	61
Figure 2.3	Phylogenetic analysis of fungi isolated from the guano of	62

frugivores bat was performed based on ITS1 and ITS5 gene data using neighbour joining (NJ) with bootstrap value 1000 replications. *Mucor indicus* strainCCUG22424SSH were included as out group in ITS phylogeny.

Figure 2.4 Colony morphology of fungi isolated from the guano of bats (A) Control, (B) *Aspergillus versicolor* strain PKM16, (C) *A. versicolar gr.*, (D) *Aspergillus versicolor* strain PKM25, (E) *A. flavus*, (F) *Aspergillus flavus* strain PKM24, (G) *Aspergillus flavus* isolate VE7, (H) *Aspergillus flavus* strain PKY2, (I) *Aspergillus flavus* isolate pkm3, (J) *Aspergillus flavus* isolate pkm11, (K) *Aspergillus flavus* strain PKM15, (L) *Aspergillus flavus* strain PKM23, (M) *Aspergillus flavus* strain PKM18, (N) *Aspergillus flavus* strain VE1, (O) *Aspergillus flavus* isolate pkm2 and (P) *Aspergillus oryzae* isolate VE9. 63

Figure 2.5 Light micrographs of fungi isolated from the guano of (A) *A. versicolor* strain PKM16, (B) *A. versicolar gr.*, (C) *A. versicolor* strain PKM25, (D) *A. flavus*, (E) *A. flavus* strain PKM24, (F) *A. flavus* isolate VE7, (G) *A. flavus* strain PKY2, (H) *A. flavus* isolate pkm3, (I) *A. flavus* isolate pkm11, (J) *A. flavus* strain PKM15, (K) *A. flavus* strain PKM23, (L) *A. flavus* strain PKM18, (M) *A. flavus* strain VE1, (N) *A. flavus* isolate pkm2 and (O) *A. oryzae* isolate VE9. 64

Figure 2.6 Scanning Electron micrographs of fungi isolated from the guano (A) *Aspergillus versicolor* strain PKM16, (B) *A. versicolar gr.*, (C) *A. versicolor* strain PKM25, (D) *A. flavus*, (E) *A. flavus* strain 65

PKM24, (F) *A. flavus* isolate VE7, (G) *A. flavus* strain PKY2, (H) *A. flavus* isolate pkm3, (I) *A. flavus* isolate pkm11, (J) *A. flavus* strain PKM15, (K) *A. flavus* strain PKM23, (L) *A. flavus* strain PKM18, (M) *A. flavus* strain VE1, (N) *A. flavus* isolate pkm2 and (O) *A. oryzae* isolate VE9.

Figure 2.7 Colony morphology of fungi isolated from the guano of bats (A) Control, (B) *Aspergillus oryzae* isolate pkm8, (C) *A. stellatus* isolate VE6, (D) *Aspergillus stellatus* isolate Pkm12, (E) *A. sclerotiorum* isolate pkm9, (F) *A. niger*, (G) *A. sydowii* strain PKY1, (H) *Aspergillus caelatus* isolate pkm10, (I) *Aspergillus* sp. PKM17, (J) *Aspergillus* sp. PKM22, (K) *Aspergillus* sp. Ambedkar, (L) *Penicillium citrinum* isolate pkm1, (M) *Penicillium citrinum* isolate pkm6, (N) *Penicillium oxalicum* isolate VE11 and (O) *Penicillium* oxalicum isolate pkm7. 66

Figure 2.8 Light micrographs of fungi isolated from the guano of (A) *Aspergillus oryzae* isolate pkm8, (B) *Aspergillus stellatus* isolate VE6, (C) *Aspergillus stellatus* isolate Pkm12, (D) *Aspergillus sclerotiorum* isolate pkm9, (E) *A. niger*, (F) *Aspergillus sydowii* strain PKY1, (G) *Aspergillus caelatus* isolate pkm10, (H) *Aspergillus* sp. PKM17, (I) *Aspergillus* sp. PKM22, (J) *Aspergillus* sp. Ambedkar, (K) *Penicillium citrinum* isolate pkm1, (L) *P. citrinum* isolate pkm6, (M) *P. oxalicum* isolate VE11 and (N) *P. oxalicum* isolate pkm7. 67

Figure 2.9 Scanning Electron micrographs of fungi isolated from the guano (A) *A oryzae* isolate pkm8, (B) *A stellatus* isolate VE6, (C) *A* 68

stellatus isolate Pkm12, (D) *A sclerotiorum* isolate pkm9, (E) *A. niger*, (F) *A sydowii* strain PKY1, (G) *A caelatus* isolate pkm10, (H) *Aspergillus* sp. PKM17, (I) *Aspergillus* sp. PKM22, (J) *Aspergillus* sp. Ambedkar, (K) *Penicillium citrinum* isolate pkm1, (L) *P citrinum* isolate pkm6, (M) *P oxalicum* isolate VE11 and (N) *P oxalicum* isolate pkm7.

Figure 2.10 Colony morphology of fungi isolated from the guano of bats (A) Control, (B) *P. crustosum* strain PKM19, (C) *P. polonicum* isolate Pkm13, (D) *P. capsulatum* isolate pkm14, (E) *P. funiculosum*, (F) *P. concentricum* isolate VE4, (G) *P. rubidurum* isolate pkm5, (H) *Penicillium* sp., (I) *Penicillium* sp., (J) *Cladosporium tenuissimum* isolate VE8, (K) *C cladosporiodes*, (L) *C. resinae*, (M) *Cladosporium* sp. VE3, (N) *Alternaria tenuissima* strain NKG1, (O) *Alternaria* sp. NKG2 and (P) *Mucor indicus* isolate BBAU. 69

Figure 2.11 Light micrographs of fungi isolated from the guano of (A) *P. crustosum* strain PKM19, (B) *P. polonicum* isolate Pkm13, (C) *P. capsulatum* isolate pkm14, (D) *P. funiculosum*, (E) *P. concentricum* isolate VE4, (F) *P. rubidurum* isolate pkm5, (G) *Penicillium* sp., (H) *Penicillium* sp., (I) *Cladosporium tenuissimum* isolate VE8, (J) *C. cladosporiodes*, (K) *C. resinae*, (L) *Cladosporium* sp. VE3, (M) *Alternaria tenuissima* strain NKG1, (N) *Alternaria* sp. NKG2 and (O) *Mucor indicus* isolate BBAU. 70

Figure 2.12 Scanning Electron micrographs of fungi isolated from the guano 71

(A) *P. crustosum* strain PKM19, (B) *P. polonicum* isolate Pkm13, (C) *P. capsulatum* isolate pkm14, (D) *P. funiculosum*, (E) *P. concentricum* isolate VE4, (F) *P. rubidurum* isolate pkm5, (G) *Penicillium* sp., (H) *Penicillium* sp., (I) *Cladosporium tenuissimum* isolate VE8, (J) *C. cladosporiodes*, (K) *C. resinae*, (L) *Cladosporium* sp. VE3, (M) *Alternaria tenuissima* strain NKG1, (N) *Alternaria* sp. NKG2 and (O) *Mucor indicus* isolate BBAU.

Figure 2.13 Colony morphology of fungi isolated from the guano of bats (A) Control, (B) *Mucor* sp., (C) *Chrysosporium tropicum*, (D) *Paecilomyces varitii*, (E) *Absidia corymbifera*, (F) *Sarocladium implicatum* isolate pkm4, (G) *Malbranchea* sp., (H) *Trichoderma sp*, (I) *Periconia* sp. VE2, (J) *Trichocomaceae* sp. PKM21, (K) *Davidiella* sp. PKM20 and (L) *Yeast*. 72

Figure 2.14 Light micrographs of fungi isolated from the guano of (A) *Mucor* sp., (B) *Chrysosporium tropicum*, (C) *Paecilomyces varitii*, (D) *Absidia corymbifera*, (E) *Sarocladium implicatum* isolate pkm4, (F) *Malbranchea* sp., (G) *Trichoderma* sp., (H) *Periconia* sp. VE2, (I) *Trichocomaceae* sp. PKM21, (J) *Davidiella* sp. PKM20 and (K) *Yeast*. 73

Figure 2.15 Scanning Electron micrographs of fungi isolated from the guano (A) *Mucor* sp., (B) *Chrysosporium tropicum*, (C) *Paecilomyces varitii*, (D) *Absidia corymbifera*, (E) *Sarocladium implicatum* isolate pkm4, (F) *Malbranchea* sp., (G) *Trichoderma* sp., (H) *Periconia* sp. VE2, (I) *Trichocomaceae* sp. PKM21, (J) 74

Davidiella sp. PKM20 and (K) *Yeast*.

Figure 3.1 FTIR absorbance spectra of guanophilic fungi such as *A. flavus* isolate pkm2 (1), *A. flavus* strain PKM18 (2), *A. flavus* isolate pkm11 (3) and *A. flavus* strain PKM24 (4). 89

Figure 3.2 FTIR absorbance spectra of guanophilic fungi such as *A. flavus* isolate VE7 (5), *A. flavus* (6), *A. versicolor* strain PKM16 (7) and *A. versicolor* strain PKM25 (8). 90

Figure 3.3 FTIR absorbance spectra of guanophilic fungi such as *A. versicolar gr.* (9), *A. oryzae* isolate pkm8 (10), *A. oryzae* isolate VE9 (11) and *A. sydowii* strain PKY1 (12). 91

Figure 3.4 FTIR absorbance spectra of guanophilic fungi such as *A. stellatus* isolate Pkm12 (13), *A. stellatus* isolate VE6 (14), *A. stellatus* isolate VE6 (15) and *A. sclerotiorum* isolate pkm9 (16). 92

Figure 3.5 FTIR absorbance spectra of guanophilic fungi such as *A. niger* (17), *Aspergillus* sp. PKM22 (18), *Aspergillus* sp. PKM17 (19) and *P. funiculosum* (20). 93

Figure 3.6 FTIR absorbance spectra of guanophilic fungi such as *P. citrinum* isolate pkm6 (21), *P. oxalicum* isolate pkm7 (22), *P. capsulatum* isolate pkm14 (23) and *P. concentricum* isolate VE4 (24). 94

Figure 3.7 FTIR absorbance spectra of guanophilic fungi such as *P. oxalicum* isolate VE11 (25), *P. rubidurum* isolate pkm5. (26), *Penicellium* sp. (27) and *C. cladosporiodes* (28). 95

Figure 3.8 FTIR absorbance spectra of guanophilic fungi such as *C. resinae* (29), *C. tenuissimum* isolate VE8 (30), *C. tropicum* (31) and *P.* 96

varitii (32).

Figure 3.9 FTIR absorbance spectra of guanophilic fungi such as *A. corymbifera* (33), *Cladosporium* sp. VE3 (34), *Davidiella* sp. PKM20 (35) and *Trichocomaceae* sp. PKM21 (36). 97

Figure 3.10 FTIR absorbance spectra of guanophilic fungi such as *Periconia* sp. VE2 (37), *M. indicus* isolate BBAU (38), *Mucor* sp. (39) and *Malbranchea* sp. (40). 98

Figure 3.11 FTIR absorbance spectra of guanophilic fungi such as *Trichoderma* sp. (41) and *Yeast* (42). 99

Figure 4.1 Light microscope images of food remnants: Legs of Hymenoptera (A, B and C), Coleoptera (D), elytra of Coleoptera (E), Odonata (F and G) and Orthoptera (H and I), wing of Neuroptera (J and K), wing of Hymenoptera (L and M), Coleoptera (N), leg of Orthoptera (O), legs of Diptera (P), leg of Odonata (Q), feather of a bird (R), wing of Hymenoptera (S), Elytra of Coleoptera (T), Legs of Hymenoptera (U), Orthoptera legs (V) Hymenopteran wing (W), Coleopteran antenna (X - Z), mandible of ant (AA) and feather (AE). 114

Figure 4.2 Scanning Electron Microscope images of food remnants: Leg of Hymenoptera (A), beetle (B), antenna of Coleoptera (C), legs of Hymenoptera (D and E), leg of Coleoptera (F), leg of Hymenoptera (G), leg of Coleoptera (H – J), whole body of Coleoptera (K), leg of Coleoptera (L – N), leg of Diptera (O – Q), wing of Odonata (R), leg of Orthoptera (S), leg of Lepidoptera (T), antenna of Coleoptera (U – V), feather of bird 115

	(W), feather of bird (W), Coleoptera Abdominal (X) and legs Coleoptera (Y, and Z), Orthoptera legs (AA), Orthoptera wing (AB), Hymenopteran legs (AC and AD), Coleopteran antenna (AE), Legs of Hymenoptera (AG - AJ), Coleoptera wing (AK), Coleoptera antenna (AL), Coleoptera elytra (AM), Coleoptera (AN) and Orthoptera (AO and AP).	
Figure 4.3	SEM-EDS spectra of *R. hardwickii* guano samples show the presence of elements such as Cl, K, Ca, Na, S, Fe, Mg, Al and P.	116
Figure 4.4	SEM-EDS spectra of *R. microphyllum* guano samples show the presence of elements such as Fe, K, Ca, Al, Mg, Na, S, Zr, Tnd Ti.	117
Figure 4.5	SEM-EDS spectra of *S. heathii* guano samples show the presence of elements such as Cl, K, Na, S, Al and P.	118
Figure 4.6	SEM-EDS spectra of *S. kuhlii* guano samples show the presence of elements such as Cl, K, Na, S, Fe, Mg, Al and P.	119
Figure 4.7	SEM-EDS spectra of *P. coromandra* guano samples show the presence of elements such as K, Ca, Na, S, Fe, Mg, Al and P.	120
Figure 4.8	SEM-EDS spectra of *T. nudiventriss* guano samples show the presence of elements such as K, Ca, Na, S, Fe, Mg, Al and P.	121
Figure 4.9	SEM-EDS spectra of *M. lyra* guano samples shows the presence of elements such as Cl, K, Ca, Na, S, Fe, Mg, Al and Ti.	122
Figure 4.10	SEM-EDS spectra of *H. fulvus* guano samples show the presence of elements such as Cl, K, Ca, Na, S, Fe, Mg, Al and P.	123

ABBREVIATIONS

%	-	Percentage
ºC	-	Degree Celsius
º	-	Degree
h	-	Hours
mm	-	Millimetre
g	-	Gravitational force
cm	-	Centimetre
ng	-	Nano gram
µl	-	Microliter
KV	-	Kilo volt
KBr	-	Potassium bromide
mg	-	Milligram
kg	-	Kilogram
sec	-	Second
v	-	Variable
s	-	Strong
m	-	Medium
sh	-	Sharp
w	-	Weak
SEM	-	Scanning Electron Microscope
FTIR	-	Fourier Transform Infrared Spectrophotometer

General Introduction

Bats are the second largest group of mammals after rodents, divided into two suborders the Megachiroptera (Frugivorous bats) and the Microchiroptera (Insectivorous bats). More than 1,300 species of bats are reported globally (BCI, 2016).

Bats spend more than half of their lives in roost (Kunz and Pierson 1994), therefore selection of diurnal roost influence the survival of bats (Barclay and Kurta, 2007). For selecting suitable roosts many factors are important such as microclimate, structural characteristics of roost, surrounding habitat, disturbance by humans and risk of predation. Bats use many kinds of roosts including caves, mines, building crevices, tree cavities and various other human made structures such as unused building, historical monuments.

Bats play exceptional role in ecological balance, nutrient cycling and redistribution of forests. Insectivorous bats consume a large quantity of insects every night (Whitaker, 1995). Due to high metabolic rate of insectivorous bats during flight bat require a large amount of Michell (Altringham, 1996) that's why they are called voracious feeders of nocturnal insects including many crop and forest pests. The *Rhinopoma mirophyllum umkinneari* feeds on some of the most noticeable insect pests of various crops and controlling the destructive insect population in the natural crop ecosystem. *Megaderma lyra* are gleaners on large insects and small vertebrates such as frogs, mice, fish, and geckoes (Advani, 1981) from ground and water surfaces.

Bat guano with high humidity along with constant temperature through the year, provides optimal environmental conditions, ideal for fungal growth. Guano from sangivorous bats is typically inhabited by fly larvae, springtails and beetles. That's why bat guano play important role for fungal growth.

Guanophilic fungi of bats usually serve as saprotrophs or pathogens or as transient chemo heterotrophic microorganisms (Northup et al., 1997). Insectivorous bats are known to be the prime contenders as reservoirs of medically important fungi. The incidence of histoplasmosis being transmitted from bat droppings (guano) to humans occurs infrequently. Nevertheless, fresh bat droppings can contain the histoplasmosis fungus. White fungus (*Geomyces destructans*) is a psychrophilic (cold-loving) fungus that cause white nose syndrome in bats, fungus grows on the face, ears, or wings of affected bats.*Geomyces destructans* is closely related to *G. pannorum*, which causes skin infections in humans.

Due to the culture limitations, misidentifications in culture collections, and unexplored habitats, only about 5% of fungal species have been accurately described (Hawksworth and Rossman, 1997). Molecular methods based on DNA analysis can reveal fungal diversity in ecosystems, and offer the potential benefits of highly sensitive and rapid detection (Saad *et al*., 2004). The molecular identification of fungi to species level has been based mostly on the use of variable ribosomal-DNA (rDNA) internally transcribed spacer (ITS) regions.

The essential feature of the FTIR analysis was to determine the spectra which would permit to differentiate the various species based on their spectra. Some Yeast and Dermatophytes were identified and grouped by using FTIR spectroscopy (Salem *et al*., 2010). Six species of *Candida* were also identified by FTIR spectroscopy (Essendoubia *et al*., 2005) thus FTIR microspectroscopy a promising clinical approach, because compared to conventional and molecular techniques, it is time and money saving, has great identification and discriminating potentials, and is amenable to an automated high-throughput routine system. It can be improved further in terms of reliability.

There were very little information is available on the guanophilic fungus of bats. Here I made an attempt to elaborate some knowledge on the guanophilic fungus. The guanophilic fungus of bats were isolated and characterize by morphological, molecular and Fourier Transform Infrared method.

Review of Literature

Microclimate of bats

Bats use roost sites to reduce energy costs, for reproduction, and to protect themselves from harsh weather conditions and predators. Selecting the right roost is important, because roost quality, e.g. microclimatic condition, influences survival and reproduction in bats.

Tidemann and Flavel (1987) found that tree-hole bats were adaptable enough to roost in cavities in man-made structures if these approximate natural hollows. They found that the *Eptesicus vulturnus*, *Chalinolobus morio*, *Nyctophilus geoffroyi*, *N. gouldi* and *Mormopterus planiceps* selects roosts with entrances and cavities having one dimension not much larger than themselves. None of them were far from water and, although there were significant differences between species values of some roost attributes, the similarities between them are probably more important. A high degree of variability in most measured attributes suggests that none of these species is highly selective.

Sedgeley (2001) observed that the New Zealand long-tailed bat *Chalinolobus tuberculatus* selects roosts in small knot-hole cavities with specific structural properties relative to available cavities. They found that the microclimate with ambient conditions, roost and available knot-hole cavities had stable microclimates displaying only small ranges in temperature and humidity. Roost cavities had higher minimum temperatures, and maximum temperatures occurred significantly later in the day and continued for significantly longer. Temperature inside cavities was lower than ambient temperature in the day and was warmer (and peaked) at night. Mean temperatures within trunk-hollows (not known to be used by *C. tuberculatus*) were cooler than mean ambient and

roost temperatures, and temperature ranges in hollows were large and fluctuated similarly to ambient temperatures. Humidity ranges were less and high humidity was maintained for longer. The results suggest that *C. tuberculatus* selects maternity roost sites with microclimatic conditions that are likely to occur for substantial energetic benefits.

Kerth *et al*. (2001) examined and tested the roost choice of 21 individually marked female *Myotis bechsteinii* living in one maternity colony. Roost occupancy over 160 census days reflected significant temperature differences and preferences changed with the season. Females significantly preferred cold roosts before parturition, whereas postpartum, significantly favoured warm roosts. Temperature preferences were independent of the roost site, and thus roost selection was based directly on temperature. Accessing too many roosts provide different microclimates that is likely to be important for successful reproduction in the endangered Bechstein's bat.

Salgado and Pérez-torres (2015) studied the characteristics of roost sites used by *Carollia perspicillata* in a cave with an area of approximately 600 m^2 located in a remnant of tropical dry forest in Colombia. From 156 roost sites, seven types of roost, the Cavity-with-Protrusions, Cavity with Crack and Protrusions, and Cavity types were not used by *C. perspicillata*. The results indicate that the selection of the roost sites was addressed by the tendency of lower values, environmental characteristics, and spatial measures.

Hutchinson and Lacki (2001) found that the avoidance of diurnal predators is considered a primary selection factor of roosting sites by red bats (*Lasiurus borealis*), as this species is cryptically coloured and difficult to observe in diurnal roosts. The results indicate that roosting sites of red bats had significantly lower overall average

temperatures, overall variance in temperatures, average diurnal temperatures, and variance in diurnal temperatures than other locations in the habitat, with no difference observed for any nocturnal temperature variable. These results suggest that Red Bats roosting in forest habitats may gain a thermal advantage by selecting diurnal roosting sites that ameliorate variability and extremes in temperature.

Guanophilic fungus

The guano of bats contains medically significant fungi. Bat guano acts as good substrate for fungal growth and offers optimal environmental conditions. The fungi commonly serve as saprotrophs and/or pathogens or as transient chemo heterotrophic microorganisms.

Mok *et al*. (1982) captured 2,886 bats in the Amazon basin of Brazil for the isolation of fungi. They collected livers, spleens, and lungs of 155 bats (5.4%), in which they found 186 fungal isolates of the genera *Candida* (123 isolates), *Trichosporon* (26 isolates), *Torulopsis* (25 isolates), *Kluyveromyces* (11 isolates), and *Geotrichum* (1 isolate). *Candida parapsilosis*, *C. guilliermondii*, *C. albicans*, *C. stellatoidea*, *C. pseudotropicalis*, *Trichosporon beigelii*, and *Torulopsis glabrata* are a pathogenic species. They found that fungal isolation frequency varied from one to three and the mycoflora diversity varied from seven to 16 fungi. They isolated 38 fungal species, in which 36 not previously reported as in vivo bat isolates. Out of the 27 culture-positive bat species, 21 had not been previously described as mammalian hosts for medically or nonmedically important fungi.

Hashemi and Emami (2003) conducted an experiment for the isolation of *Histoplasma* in central Asia because earlier clinical cases of histoplasmosis have been reported from many parts of the world principally from the USA and to a lesser extent

from central and South America. Some sporadic cases are recorded in medical literature from Europe, Africa and Asia. *Histoplasma capsulatum* are etiologic agent of histoplasmosis and cosmopolitan geophilic fungus associated with avian and chiroptera. The association of bats with cases of histoplasmosis in man and the isolation of *H. caplulatum* from organs and faeces of naturally infected bats suggest that certain species of chiroptera may play a role in the epidemiology of histoplasmosis. For this purpose they examined 800 bats representing three genus include *Myotis*, *Rhinolophus* and *Sherbersi* from during 1994 to 1995 (12 months) from 2 caves in the North and West in Iran. However *Histoplasma capsulatum* was not recorded.

Martin and Rygiewicz (2005) review on the importance of internal transcribed spacer (ITS) regions of fungal ribosomal DNA (rDNA) and highly variable sequences of ITS are great importance in distinguishing fungal species by PCR analysis. Previously published PCR primers available for amplifying these sequences from environmental samples provide varying degrees of success at discriminating against plant DNA while maintaining a broad range of compatibility. Typically, it has been necessary to use multiple primer sets to accommodate the range of fungi under study, potentially creating artificial distinctions for fungal sequences that amplify with more than one primer set. A nested set of 4 primers was developed that reflected these criteria and performed well amplifying ITS regions of fungal rDNA. Primers in the 5.8S sequence were also developed that would permit separate amplifications of ITS1 and ITS2. A range of Basidiomycete fruiting bodies and Ascomycete cultures were analyzed with the nested set of primers and Restriction Fragment Length Polymorphism (RFLP) fingerprinting to demonstrate the specificity of the assay. Single ectomycorrhizal root tips were similarly analyzed. These primers have also been successfully applied to Quantitative PCR (QPCR), Length Heterogeneity PCR (LH-

PCR) and Terminal Restriction Fragment Length Polymorphism (T-RFLP) analyses of fungi. A set of wide-range plant-specific primers were developed at positions corresponding to one pair of the fungal primers. These were used to verify that the host plant DNA was not being amplified with the fungal primers. These plant primers have been successfully applied to PCR-RFLP analyses of forest plant tissues from above and below ground samples and work well at distinguishing a selection of plants to the species level. The complete set of primers was developed with an emphasis on discrimination between plant and fungal sequences and should be particularly useful for studies of fungi where samples also contain high levels of background plant DNA, such as verifying ectomycorrhizal morphotypes or characterizing phylosphere communities.

Sajid *et al*. (2006) carried out an experiment to determine the incidence of *Aspergillus fumigatus* in commercial poultry flocks. They isolated *A. fumigatus* from the lungs and air sacs of affected birds. They took sixty four suspected cases of fungal infection in which thirty one cases were found positive for *A. fumigatus*. They found after post-mortem examination gross lesions in lungs, trachea and thoracic air sacs. Small white nodular lesions 1 mm diameter was observed in the congested lungs and thoracic air sacs. They found branched septate hyphae under oil emersion and cultured on sabouraud agar. Velvety white colonies were seen, at first, which turned dark green to grayish later. They concluded that that incidence of *A. fumigatus* was considerably high in flocks reared on sawdust litter as compared to rice husk litter.

Balajee *et al*. (2007) reviewed that the so many studies on use of traditional morphological method for the identification of clinically relevant aspergilli species had limited utility. The use of comparative sequence based methods with traditional method can offer better resolution of species within this genus.

Guan *et al*. (2007) reidentified an isolate of *Aspergillus* in Heilongjiang Province, China earlier its name was *Aspergillus* sp. D-1. After the examination of its morphology and conidial ornamentation under scanning electron microscopy, the alignments and phylogenetic relationship on the basis of ITS-5.8S gene sequences were studied. The evidence from the morphology and ITS-5.8S DNA sequences suggest that *Aspergillus* sp. D-1 belongs to the species of *A*. versicolorand accordingly was named *A. versicolor* D-1. *Aspergillus versicolor* D-1 has the ability to reduce the *γ*, *δ*-double bond of securinine.

Houbraken (2010) examined *Penicillium citrinum* and related species using a combination of partial β-calmodulin and ITS sequence data, extrolite patterns and phenotypic characters. They described two new species *P. hetheringtonii* sp. nov.,*P. tropicoides* sp. nov., and the combination *P. tropicum* proposed. They described differences between *P. hetheringtonii* was closely related to *P. citrinum* and differs in having slightly broader stipes, metulae in verticils of four or more and the production of an uncharacterized metabolite.

Leonardo *et al*. (2012) isolated and identified fungi in guano of haematofagous and insectivores bats in not touristic hall of Lapinha´s cave- MG. They collected soil samples (1g) from three points by removing mechanically a layer of 5 cm of soil and subjected to serial dilutions in sterile deionized water to a final volume of 10 ml. These dilutions were plated on Sabouraud agar and incubated at 23°C for 1-15 days. They grouped isolated filamentous fungi colonies according to their macromorphology and confirmed by molecular (PCR), using primers directed to microsatellite region (GTG)´5. Based on the electrophoretic profile of amplimers isolates that presented the same pattern of bands, one of each group, will be selected for identification by sequencing of the internal transcribed spacer region of ribosomal DNA (ITS1 -ITS4).

Characterization of guanophilic fungus by FTIR

The use of FTIR has significantly improved in various fields such as, for the identification of bacteria, Yeasts, fungi, applied microbiological research, physiological properties. The use of FTIR is an analytical approach, non-destructive, and dynamic method. The essential feature of the FTIR analysis is to determine the spectra which would permit to differentiate the various species due to the weak variations of the cell composition which are due to changes in culture conditions (culture time, medium composition).

Essendoubia *et al*. (2005) identified *Candida* using Fourier transform infrared micro spectroscopy (FTIRM). FTIR spectroscopy is a whole-cell ''fingerprinting'' method by which they identified 6 species (*Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. kefyr*) from a collection of 57 clinical strains of Candida, isolated from hospitalised patients. Data obtained on 10 to 18 h-old microcolonies were compared to cultures of 24 h. the results clearly shown by the efficiency and the robustness of FTIR (micro) spectroscopy in identifying species with Essendoubia a classification rate of 100% for both microcolonies and 24-h cultures. FTIR microspectroscopy is thus a promising clinical approach, because compared to conventional and molecular techniques, it is time and money saving, has great identification and discriminating potentials, and is amenable to an automated high throughout routine system.

Naumann *et al*. (2005) used FTIR microscopy to detect and discriminate the wood decaying fungi *Trametes versicolor* and *Schizophyllum commune* in experimentally infected beech wood blocks. FTIR microscopy combined with a focal plane array detector and image analysis, the distribution of fungal mycelium in wood

was locally resolved and semi quantitatively recorded. Species-specific clustering of spectra of fungal mycelium grown on the wood surface and inside vessel lumina demonstrated the potential of FTIR microscopy to discriminate among fungal species decaying wood.

Kümmerle (2007) used computer based Fourier-transform infrared spectroscopy (FTIR) to identify food-borne, predominantly fermentative Yeasts and used dried Yeast suspensions films for FIR measurement. They used a reference spectrum library of 332 defined Yeast strains from international Yeast collections and their own isolates. They identified all strains of Yeast with conventional methods using physiological and morphological characteristics. They identified 722 unknown Yeast isolates both by classical methods as well as FTIR spectra with those of the reference spectrum library. By FT-IR 97.50% isolates were identified correctly. Use of FTIR is easy handling, rapid identification within 24 h when starting from a single colony, and a high differentiation capacity thus render this technology clearly superior to other routine methods for the identification of Yeasts.

Movasaghi *et al*. (2008) reviewed some of the recent advances on FTIR spectroscopy in areas related to natural tissues and cell biology. It was the second review publication resulting from a detailed study on the applications of spectroscopic methods in biological studies and summarizes some of the most widely used peak frequencies and their assignments. The aim of these studies was to prepare a database of molecular fingerprints, which will help researchers in defining the chemical structure of the biological tissues introducing most of the important peaks present in the natural tissues. In spite of applying different methods, there seems to be a considerable similarity in defining the peaks of identical areas of the FTIR spectra. As a result, it is believed that preparing a unique collection of the frequencies encountered in FTIR

spectroscopic studies can lead to significant improvements both in the quantity and quality of research and their outcomes. This article is the first review of its kind that provides a precise database on the most important FTIR characteristic peak frequencies for researchers aiming to analyze natural tissues by FTIR spectroscopy and will be of considerable assistance to those who were focusing on the analysis of cancerous tissues by FTIR spectroscopy.

Salem *et al.* (2010) made an attempt to verify the practical applicability of Fourier transform infrared (FTIR) technique in identification of some Yeast and dermatophyte species. Traditional identification systems of Yeasts and dermatophytes have many disadvantages. They had taken fifty patients with superficial fungal infections for mycological studies by culture on Sabouraud-dextrose agar with chloramphenicol and cycloheximide (actidione). Only 7 species were isolated from the 50 patients and were categorized as Yeasts - *Candida albicans* (20 isolates), *Rodotorula rubra* (2 isolates) and Dermatophytes *Microsporum canis* (10 isolates), *Trichophyton rubrum* (6 isolates), *T. violaceum* (6 isolates), *T. verrocosum* (4 isolates) and *Epidermophyton floccosum* (2 isolates). The isolates were studied using FT-IR microspectroscopy. They found spectra of the same species were identical while spectra of different species did not show similarity. This study showed that FT-IR microspectroscopy approach is promising and allows, with a single measurement, to have a ‘‘molecular fingerprint” of Yeasts and dermatophytes species.

Lecellier *et al*. (2014) conducted an experiment for routine identification offungi by using Fourier transform infrared (FTIR) spectroscopy. They identified one hundred and thirty-one strains using DNA sequencing by conventional methods. They reduced culture time of 48 h as compared to current conventional methods. Identification levels of 98.97% and 98.77% achieved were correctly assigned to the genus and species levels

respectively by using chemometric method, Partial least square discriminant analysis. FTIR spectroscopy with its high discriminating power and rapidity therefore shows strong promise for routine fungal identification. Upgrading of our database is on going to test the technique's robustness.

Diet analysis of insectivorous bats

Insectivorous bats consume a large quantity of insects every night due to high metabolic rate that's why they are called voracious feeders of nocturnal insects including many crop and forest pests. The feeding habits of insectivorous bats are of great interest to people, because they are considered to be important in the control of insects ranging from agricultural pests such as moths to disease vectors such as mosquito.

Swift *et al*. (1985) determined diet of pipistrelle bats by faecal analysis and compared with their representation in suction trap samples during pregnancy and lactation. They found that *Pipistrelles* fed mainly on Nematocera and Trichoptera and the composition of their diet reflected the availability and abundance of these insects over a wide range. Ephemeroptera and Neuroptera were significantly over represented in the diet and Nematocera, Coleoptera and Lepidoptera were significantly under represented. They reported that there is no evidence that pipistrelles selected their diet on the basis of insect size, neither in between sexes nor between pregnant and lactating females.

Leeand Lee (2005) studied the composition and seasonal variation in the diet of Japanese pipistrelles, *Pipistrellus abramus*, from fecal samples (May 1988 to June 1990) in Chutung, northern Taiwan. They found that diet of pipistrelles bats contained 12 orders of insect and spiders. The majority of pellets examined (86.3%) contained 3

to 6 prey items. The result showed that, the beetles, dipterans, hymenopterans, caddis flies, moths, true bugs, and homopterans (decreasing order) were the most frequently found in the feces. The dietary heterogeneity index (DHI) of Japanese pipistrelles was 9.25, but this varied among monthly samples, and they found no apparent seasonal patterns. They found that the diets of the bats over the 3 seasonal periods were similar, with only minor variations. Hymenopterans, moths, and caddis flies were more frequently taken, while true bugs and homopterans less frequently taken by bats after mid-July. Bats appeared to consume higher proportions of homopterans in the 1st than in the 3rd period, but higher proportions of true bugs in the 2nd than in either the 1st or 3rd periods, whereas higher proportions of hymenopterans were consistently taken in the 1st sampling year.

Aguiar and Antonini (2008) examined food habits of Vespertilionidae bats *Myotis nigricans* and *Eptesicus furinali* by fecal analysis. They found 20 fecal samples in which seven were of *E. furinalis* and 13 of *M. nigricans*. The diet of *E. furinalis* included six orders of insects: Coleoptera, Lepidoptera and Hymenoptera, Diptera, Hemiptera and Homoptera. The diet of *M. nigricans* included all the main orders consumed by *E. furinalis* and one other order Orthoptera.

Thomas *et al*. (2012) studied diet and feeding relations of six species of bats at five locations in New Hampshire and Massachusetts to improve understanding of foraging niche differentiation. They collected faecal samples from 100 big brown bats (*Eptesicus fuscus*), 154 little brown myotis (*Myotis lucifugus*), 49 northern myotis (*M. septentrionalis*), 54 eastern small-footed myotis (*M. leibii*), 9 eastern red bats (*Lasiurus borealis*), and one hoary bat (*L. cinereus*) netted during non-hibernation periods from 2004 to 2008 at four locations in Southern New Hampshire and one in north-central Massachusetts. They recorded that the beetles (Order Coleoptera) were

the major food of *E. fuscus* (mean percentage volume = 81.6%, 97% occurrence) followed by moths (Lepidoptera), with scarabaeid and carabid beetles the most abundant consumed families by volume and frequency. Moths were the most important item by volume and frequency preyed on by the remaining species.

Moosman *et al*. (2012) studied on diet of insectivorous bats *E. fuscus* and *M. lucifugus* relative to climate and richness of bat communities They examined diet of the North American big brown bats *(E. fuscus)* and little brown bats *(M. lucifugus)* using published works and their own data from New England to better understand intraspecific dietary variation and to test whether diet corresponded with climate (an indicator of availability of prey) and species richness of bat communities (a measure of potential interspecific competition). They found that diet of *E. fuscus* and *M. lucifugus*varied with climate in a manner that generally corresponded with longitude. *Eptesicus fuscus* increased use of moths in regions with cool arid summers (Western North America), whereas both species of bat consumed more beetles in moisture summer climates associated with Eastern North America. *E. fuscus* consumed fewer beetles and more moths and true flies in more diverse bat communities.

A study was carried out by Shetty and Sreepada, (2013) in Karkala D.K. district of Karnataka from November 2009 to October 2010 in a maternity colony in an abandoned house that is surrounded by coconut plantations and paddy fields. In all seasons the scales of fishes, legs of frogs and hair, tooth and legs of rats were found and included under Vertebrates. They found out of 15 insect order Coleopterans formed the major food items in all the three seasons and Vertebrates along with Coleopterans formed major food items during monsoon and postmonsoon. The work of Shetty and Sreepad conclude that microchiropteran bats play a vital role in the ecosystem and consume large volumes of insects many of which are agricultural pests and their

droppings (guano) contain large amount of partly digested insect parts. Guano of microchiropteran forms the resource base for other diverse form of consumers in the food chain including diverse microbes. These together make guano the best organic fertilizers.

Hope *et al.*(2014) studied the winter diet of natterer's bat (*Myotis nattereri*) by faecal analysis. Faecal pellets collected from a hibernation site in Southern England during two winters (December-March 2009–10 and 2010–11), indicated that *M. nattereri* forages throughout winter at least in a location with a mild winter climate. They identified seven taxonomic orders of arthropod fragments through morphological analysis, in which unexpectedly there was high proportion 67.9% of lepidopteron larvae of in the faecal pellets. From molecular analysis they identified 43 prey species from six taxonomic orders and confirmed the frequent presence of lepidopteran species that overwinter as larvae. *M. nattereri* had a wide and adaptable dietary nichebecause their diet differs from winter to other times of the year. According to Paul *et al*., the *M. nattereri* are gleaner beacuase they found high occurrence of non-volant prey suggests that gleaning allows prey capture at low ambient temperatures when the abundance of flying insects may be substantially reduced. In the diet of *M. nattereri* cutworms (lepidopteran larvae) suggests that they eats on agricultural pest species.

Ponmalar and Vanitharani (2014) conducted an experiment on insect pest management by the horse shoe bats (*Rhinolophus* species) in Kalakad Mundanthurai Tiger Reserve (KMTR), which is the home for many endemic, rare and endangered species of plants and animals, was one of the priority hotspot for conservation. Ponmalar and Vanitharani examined and identified altogether 9 insect orders from all the faecal samples of *Rhinolophus* bats. Scales and body parts of moths (Lepidoptera) were the most dominated item in all the faecal samples of all the rhinolophids followed

by Coleoptera and Diptera. This study also shows that there is high frequency of lepidopteran and coleopteran fragments in the faeces of all the four studied species. The second higher percentage of identified parts of insect orders was Coleoptera, Diptera, Hymenoptera and Hemiptera. The diet of rhinolophids showed that they also consume a major group of dipteran which includes the trill flies, mosquitoes, midges and variety of flies, leg dance flies, snipe flies, hover flies, shore flies, blow flies, dung flies, bat flies etc. They frequently selects nutrientrich abdomen of insects while discarding the wings, head, and appendages, which greatly increases feeding efficiency and the quantity of insects consumed. The dominancy and high availability of food items of insect order was Lepidoptera, Coleoptera and Diptera in summer than in winter. The other food components like hymenopterans are more in premonsoon, homopterans are more in late winter, orthopterans, hemipterans, trichopterans and neuropterans are more in postmonsoon period.

Rahman *et al*. (2015) conducted a study on habitat characteristics of *Rhinopoma hardwickii* in Charsadda district Khyber Pakhtunkhwa for a period of two years. They found a roost of *R. hardwickii* inmulberry tree, *Morus alba* within a depth of half meter, at a height of 3 meter at breast height. This tree was about 7 meter long, with 1/2 meter diameterhaving more than 70 bats, with 5 pups and 3 lactating mothers. They found that roost was located within the forest where various plant species including wild fig, *Ficus palmate*; white cedar, *Melia azedarack*; paper mulberry, *Brousonetia papyrifera*; black mulberry, *Morus nigra*, white mulberry, *Morus alba*; Indian jujube, *Zizipus mauritania*; blue gum, *Eucalyptus globules*; babul, *Acacia arabica* and commonly used as roosts by *Pteropus* giganteusat three sites. They found that the black poplar, *Populus nigra* was the most abundant, while wild fig; *Ficus palmate* were the least abundant of all species.

Elemental analysis of bat guano

The abundance of macronutrients in bat guano suggests its suitability as bio-fertilizer. Bat guano is reported to contain all the macro and micro nutrients required by plants being a good fertilizer.

Sridhar *et al*. (2006) studied on physicochemical characteristics, microflora and manure quality of guano of an endemic insectivorous cave bat *Hipposideros speoris*. They found that organic matter, total carbon, total nitrogen and phosphate were high in faecal pellets and calcium, magnesium, bacteria, actinomycetes and fungi were higher in humus like guano than faecal pellets. Physicochemical features and microbial load between faecal pellets and humus-like guano significantly differed. They found that crops, in soil amended with guano at the ratio of 20:1 showed the highest shoot length, total dry matter, nitrogen content and nitrogen uptake. The shoot length, total dry matter, nitrogen content and nitrogen uptake in both crops were significantly differed between treatment 20:1 and control. The results clearly indicated that incorporation of low amount of bat guano into the soil enhances crop production.

Chapter 01:
Roosting Ecology of Bats

Chapter 01

Roosting Ecology of Bats

Introduction

Bats are the second largest order among mammals, divided into two suborders the Megachiroptera (consists186 species in a family) and the Microchiroptera (consists 931 species in 17 families). Megachiropterans are called as Old World fruit bats and microchiropterans are insectivorous which equipped with the ability to echolocate. In India about 119 species of bats are found in nine families namely Pteropodidae, Emballonuridae, Megadermatidae, Rhinolophidae, Hipposideridae, Vespertilionidae, Miniopteridae, Rhinopomatidae and Mollosidae (Bates and Harrison, 1997).

Bats occupy a wide variety of habitats in both natural such as caves, mines, rock crevices, exposed tree branches, loose bark, buttress roots, cavities in trees, foliage, tents made from modified leaves, bridges, buildings and other manmade structures (Hinman and Snow, 2003), and they spend more than half of their lives in roost (Kunz and Pierson, 1994). Bats spend about 15 h per day at their roosting sites and therefore selection of diurnal roost influence the survival of bats (Barclay andKurta, 2007).

Many factor such as microclimate, structural characteristics of roost, surrounding habitat, disturbance by humans and risk of predation may influence roost selection by bats (Usman, 1988; Churchil, 1991; Vonhofand Barclay, 1996; Entwistle *et al*., 1997; Williams and Brittingham, 1997; Jenkins *et al*., 1998; Sedgeley and O'Donnell, 1999). Very important factors for selecting suitable roosts are availability of food, development and survival of young one (Tuttle and Stevenson, 1982), risks of predation (Fenton, 1983), social organization and energetic limits (Barbour and Dewis, 1969; Ferrara and Leberg, 2005), and reduction of thermoregulatory costs (Vaughan, 1987).

Roosts are critical habitats for bats and roost availability may limit the number and distribution of certain bat species (Humphrey, 1975). The availability of roost sites is also important in determining the diversity and abundance of bat species (Kunz, 1982). The roosting environment of bats varies among species to species and from one season to another season. The new and old world tent making bats roost in a variety of plant species in which they modify the leaves and stems into tents (Kunz, 1982; Brooke, 1987; Kunz and Pierson, 1994; Bhat and Kunz, 1995). Bats use many kinds of roosts including caves, rock crevices, tree cavities and various other human made structures such as unused building, historical monuments, temple etc and these sites typically offer bats a stable microclimate (Racey *et al*., 1987; Ransome, 1990).

Cavity size, shape and opening may also directly influence the population of bats that are present, social organization and predation risk (Kunz and Lumsden, 2003), tree cavities may also be selected as roosts for their thermal environments (Vonhof and Barclay, 1996; Sedgeley, 2001). Bats have high mass-specific metabolic rates due to their large surface area to volume ratio, which results in an increased energetic cost to maintain a high body temperature (Speakman and Thomas, 2003). Most species of bats cannot manipulate the physical structure of their roosts (Kunz, 1982), so it is necessary that selected roost may provide preferable microclimatic conditions to enhance reproductive success and survival (Humphrey, 1975). Large diameter trunks of trees provide additional cavity insulation than trees with slender trunks (Sluiter *et al*., 1973; Gellman and Zielinski, 1996), because as the diameter of a tree increases the cavity wall thickness and thermal inertia of wood also increase (Derbyand Gates, 1966; Nicolai, 1986). Thick cavity walls have been shown to reduce temperature ranges and slow temperature changes as compared to ambient temperatures (Vonhof and Barclay, 1996).

India comes under tropical country has a rich biodiversity of flora and fauna, though, the state Uttar Pradesh has a good number of natural resources like reserve forests, sanctuaries, national parks, old monuments, palaces, caves etc which harbor bats. Therefore, the present study was aimed to give information on roosting sites and microclimate of bats of Uttar Pradesh.

Materials and Methods

The field surveys were carried out at roosting sites such as historical monuments, old temples, abandoned buildings, caves, crevices, tree holes and foliage roosts periodically during the study period.

The roost characteristics such as size, shape, number of occupants and temperature, humidity, geographical locations were observed and nature of roost (manmade / natural, tree roost / building roost), roost height above ground, dimension of roost cavity (i.e. breadth and depth) were recorded for each roost type located within the study area. Night roosting activity was assessed visually using night vision Binocular. The colony size was assessed through direct count and photographic methods (Tuttle, 1979).

Results

Two species of megachiropteran bats such as *Rousettus leschenaulti* and *Cynopterus sphinx* and eight species of microchiropteran bats such as *Rhinopoma hardwickii*, *R. microphyllum*, *Scotophilus heathii*, *S. kuhlii*, *Pipistrellus coromandra*, *Megaderma lyra* and *Taphozous nudiventris* were observed at different districts of Uttar Pradesh during the study period (Table 01 and Fig. 1.1 – 1.2).

Fulvous fruit bat, *Rousettus leschenaulti* (Desmarest, 1820)

The fulvous fruit bat, *R. leschenaulti* lives in colonies which varied from few individuals to several thousands. A colony of *R. leschenaulti*consists 1500 individuals observed in an unused building at Chakravarti Mahraj Dashrath Mahal, situated near the Hanuman Gharhi. The bats were roosted on wooden roof at about 15 feet of height. There were guavavaandpapaya orchards adjacent to the roost site. Further, the roosting site was located nearer to the river Yamuna. The colony located at Raj Sadan, Ayodhya had about 291 individuals of *R. leschenaulti* (Fig. 1.1A). The average temperature and humidity of the roost sites of *R. leschenaulti* were 32 ± 00 °C and 66 ± 00 %, respectively.

The short-nosed fruit bat, *Cynopterus sphinx* (Vahl, 1797)

The short-nosed fruit bat, *C. sphinx* is a medium sized fruit bat and lives in small harem. Its diurnal roosts include the underside of the dry fronds of Kitul palms *Caryota urens*, the stems of mast tree *Polyalthiya longifolia*, banyan tree *Ficus bengalensis* and dome of abandoned buildings. The colonies of *C. sphinx* were observed in a tree hole of *F. bengalensis* and the population size ranged from 3 – 15. A colony consist 65 individuals of *C. sphinx* was observed inside a dome at Sisandi House. The dome is located at 15 feet height from the ground and open at all directions, the dome was constructed over a deep well. Unlike other roosts such as tent roost and cavity roost, all 65 individuals occupied as a cluster without forming small harems. Another colony of 5 individuals was found in *Polyathya longifolia* (Ashok) tree, the height of colony was about 6 feet from the ground. The average relative temperature and humidity of the roost sites of *C. sphinx* were 29 ± 1.41 °C and 65 ± 7.07 % respectively.

Lesser Mouse-tailed Bat, *Rhinopoma hardwickii* (Gray, 1831)

Three colonies of *R. hardwickii* were observed at different locations in the study area. A colony of *R. hardwickii* consists about 700 individuals found in a historical monument at Khusurubagh, 3 km away from the river Ganga (Fig. 1.1 C). The height of roost was 10 feet from the ground and there were four entrances to the roost. Bats were observed on the window panels of the monument. In addition, about 900 individuals of *R. hardwickii* were observed beneath the staircase of Atala mosque, the roost was located about 12 feet from the base. The average relative temperature and humidity of the roost sites of *R. hardwickii* were 29.50 ± 0.71°C and 77.50 ± 3.54 %, respectively.

Greater Mouse-tailed Bat, *Rhinopoma microphyllum* (Brunnich, 1782)

A colony of *R. microphyllum*consists 1200 individuals found in Chunar fort, Mirzapur (Fig. 1.1 D). The fort is located closes to the river Ganga. The individuals of *R. microphyllum* occupied the roof of 6 rooms (10l × 8 w × 12 h) in the abandoned fort. In addition, about 2000 individuals of *R. microphyllum* found roosting in a natural cave at Chitrakoot. The bats were roosted in small groups as well as solitarily. The cave was located amid of dense forest and the cave entrance was narrow which prevent the entry of bigger animals. Another colony of *R. microphyllum* consists3000 individuals found in Pal Kothii, Varansi which was too located near Ganga. The individuals were roosting on the walls. The average relative temperature and humidity of the roost sites of *R. microphyllum* were 31.10 ± 4.71°C and 79.67 ± 8.96 %, respectively.

Greater Asiatic Yellow House Bat, *Scotophilus heathii* (Horsfield, 1831)

The colony size of *S. heathii* ranges from 6 to 50 individuals. It lives in holes, crevices and historical monuments. A colony of *S. heathii* was found in an abandoned

building of Balister Singh (Fig. 2.2 E). The floor are of the roost was over 120 square feet and located at 12 feet height. About 40 individuals of *S. heathii* were observed in four groups. In addition, the colonies of *S. heathii* were observed in tree clefts of *Ficus bengalensis* at Allipur and Kashipur villages of Hardoi. The depth of crevices was ranged from 18 to 20 cm while the height varied from 44 to 55 cm. The average relative temperature and humidity of the roost sites of *S. heathii* were 29.93 ± 2.00° C and 67.67 ± 2.52 %, respectively.

Lesser Asiatic Yellow House Bat, *Scotophilus kuhlii* (Leach, 1821)

Scotophilus kuhlii was found in rural and urban areas of Uttar Pradesh. The roosts were found in roofs, crevices of houses, old building, caves, temples, tree hollows and dry fronds of palm trees. The population size varied from few individuals to many hundreds. In addition, the colonies of *S. kuhlii* were observed in the clefts of *F. bengalensis* and crevices of abandoned building at Hardoi (Fig. 1.1F). The height of building roost was about 15 feet. The length, width and depth of tree cleft were 36 x19 x 25 cm. Though the roosts were surrounded by human habitation but they were out of human reach. The dump of excreta observed under the tree roost showed the long existence of *S. kuhlii*. A large colony of *S. kuhlii* consists 1400 individuals found in the dry fronds of palm tree at Babasaheb Bhimrao Ambedkar University, Lucknow. The average relative temperature and humidity of the roost sites of *S. kuhlii* were 34.47 ± 1.75 °C and 75.00 ± 7.07 %, respectively.

Little Indian bat, *Pipistrellus coromandra* (Gray, 1838)

The colony size of *P. coromandra* ranges from 8 – 12 individuals. *Pipistrellus coromandra* preferred to roost in tree cavity and crevices of abandoned buildings and rocks. The roosts of *P. coromandra* were observed at 8 – 15 feet height. The building

roost of *P. coromandra* was observed at Kali Khoh temple at Chunar. The average relative temperature and humidity of the roost sites of *P. coromandra* were 28.70 ± 00 °C and 70.00 ± 00 %, respectively.

Naked-rumped Tomb Bat, *Taphozous nudiventris* (Cretzschmar, 1830)

The distribution of *T. nudiventris* in the study area was limited, only two colonies were observed. The colony size was ranged from 160 – 600 individuals. The first colony was observed at Jhansi fort at 12 feet height with 250 individuals in 12 harems. The second colony was observed in a historical monument at Bhuragrah, Banda. The colony consists of 450 individuals of *T. nudiventris* and located at 22 feet height. The average relative temperature and humidity of the roost sites of *T. nudiventris* were 27.90 ± 0.14 °C and 64.50 ± 0.71 %, respectively.

Indian False Vampire Bat, *Megaderma lyra* (Geoffroy, 1810)

A colony of *M. lyra*consists 500 individuals observed at Rafi Ahmad Inter College, Hardoi. They occupied a space below the staircase of an abandoned building. The height of the roost was 7 – 18 feet. The individuals formed small group consist of 6 – 8 individuals. The bat guano was accumulated to a thickness of 8 cm on the floor. The colony of *M. lyra* located in an abandoned fort of King Rookmangal Singh which converted into a College. A colony consists of 300 - 370 individuals,found roosting in a basement of an abandoned fort of King Rudra Pratap Singh Shahi located at Diyara, Sultanpur. The colony occupied an underground hall (8×4×5 m). The average relative temperature and humidity of the roost sites of *M. lyra* were 30.50 ± 2.12 °C and 70.00 ± 7.07 %, respectively.

Fulvus Leaf-nosed Bat, *Hipposideros fulvus* (Gray, 1838)

The roosting sites of *H. fulvus* include old building, caves and tunnels. The roosts of *H. fulvus* were observed at the basementof a historical monument, Bara Imambara, Lucknow. The average height of roost was 11.5 feet from the ground and the roosting patterns of *H. fulvus* was scattered. The colony size was ranged from 100 – 200 individuals. *Hipposideros fulvus* was not observed in harem. The average relative temperature and humidity of the roost sites of *H. fulvus* were 32.00 ± 00 °C and 66 ± 00 %, respectively.

Discussion

In the present study a total of 10 bat species such as *R. leschenaulti*, *C. sphinx*, *R. hardwickii*, *R. microphyllum*, *S. heathii*, *S. kuhlii*, *P. coromandra*, *M. lyra* and *T. nudiventris* were observed. Among the observed species in the study area, the occurrence of *C. sphinx*, *R. hardwickii*, *S. heathii*, *S. kuhlii*, *P. coromandra* and *M. lyra* was abundant and the occurrence of *T. nudiventris* was scarce.

The results of present study revealed that bats selected different types of habitats for their roosting. The insectivorous bats preferred to roost in tree cavities, wall crevices, roofs of abandoned buildings, historical monuments and caves. The roosts were observed within human habitation both in rural and urban areas. The type of habitat a flying animal chooses to live in as well as its way of exploiting the habitat is closely related to its body size, wing shape, flight style, flight speed and flight energetics (Norberg, 1990). Frugivorous bats such as *C. sphinx* were found roosting mostly in buildings in the study area, on few occasion occupied tent roosts. The number of occupants in building roosts is very high compared to the plant roosts. *Cynopterus sphinx* is a tent roosting bat, however it was observed in building roosts. Fulvous fruit

bat, *R. leschenaulti* was widely distributed in India (Bates and Harrison, 1997). The current study also revealed that roost sites of *R. leschenaulti* were stable, undisturbed, and long lasting. The roost sites of *R. leschenaulti* included caves, deserted buildings and disused tunnels, wells and temples (Phillips, 1980; Chandrashekaran and Marimuthu, 1994).

The insectivorous bats are primary consumers of nocturnal insects and play a vital role in insect control on large scale (Kunz, 1982). The insects belong to the orders Coleoptera, Lepidoptera, Homoptera and Hemiptera are the major agricultural pests (Oliveira, 2005). The outcome of this study revealed that the state Uttar Pradesh offer potential roost sites for both insectivorous and frugivorous bats. A large number of monuments and caves exits in U.P. offer stable roost sites to bats. Further, they provide favorable conditions for roosting. In addition, occurrence of larger trees also harbored both insectivores and frugivores bats in Uttar Pradesh.

Table 1.1 Roost sites, roost characteristics and microclimate of bats.

Bat sp.	Roosting sites	No. of occupants	Type of roost	Temperature (°C)	Humidity (%)
Rousettus leschenaulti	Chakravarti Mahraj Dashrath Mahal (26°47'45.49" N 82°11'47.38" E)	1500 – 1600	Building roost	32	66
	Raj Sadan, Ayodhya (26°47'31.88" N 80°12'19.98" E)	291	Building roost	32	66
Cynopterus sphinx	King Vijay Kumar Tripathi Sisandi House (26°37'18.15"N, 80°55'44.65"E)	70	Abandoned building	30	70
	BBAU campus, Lucknow (26°46'4.35"N, 80°55'34.67"E)	5 – 6	Tree roost	28	60
Rhinopoma hardwickii	Jhushi fort and Khusurubagh fort, Allahabad (25.45°N, 81.85°E)	700 – 750	Historical monument	29	80
	Atala mosque, Jaunpur (25.73°N, 82.68°E)		Historical monument	30	75
	Diyara, Sultanpur (26.45°N, 82.11°E)		Fort	30	76

Rhinopoma microphyllum	Kal Kothary, Chunar Mirzapur (25° 7'24.06"N, 82°52'32.68"E)	1100 – 1150	Fort	32	75
	Thar Ganga Ghat at Varanasi (25.28°N, 82.95°E),	2500 – 3000	Building	35.3	74
	Gupt Godavari, Chitrakoot (25° 5'52.54"N, 80°46'7.64"E)	1800 – 2000	Natural cave	26	90
Scotophilus heathii	Balister Singh, Purwa, Unnao (26°27'24.02"N, 80°46'16.76"E)	40	Building	32	68
	Allipur and Kashipur villages in Hardoi (27°25'15.65"N, 80° 4'43.09"E)	6	Tree	29.8	65
	Railway station of Hardoi (27°23'56.27"N, 80° 8'52.51"E)	8	Tree	28	70

Scotophilus kuhlii	Hardoi (27°17'52.43"N, 80°11'15.02"E)		Abandoned building	33.23	70
	BBAU campus, Lucknow	1200 – 1400	Tree	35.7	80
Pipistrellus coromandra	Mahakali Kali Khoh, Chunar (25° 9'15.28"N, 82°29'3.15"E)		Abandoned building	28.7	70
Taphozous nudiventris	Jhansi Fort, Jhansi (25°27´27.95″ N 78°34´31.62″E)	160 – 600	Historical Monument	27.8	64
	Bhuragrah fort, Banda (25°28´33.80″ N 80°18´34.91″E)	450 – 500	Historical Monument	28	65
Megaderma lyra	Rafi Ahmad Inter College, Hardoi	6 – 8	Abandoned building	32	65
	King Rudra Pratap Singh Shahi, Diyara, Sultanpur (26°13'31.86"N, 82°17'2.86"E)	300 – 370	Abandoned building	29	75
Hipposiderous fulvus	Bara Imambara, Lucknow (26°52'6.96"N, 80°54'45.52"E)	3 – 4	Historical Monument	32	65

Figure 1.1 Different species of bats observed in the study area: *Rousettus leschenaulti* (A), *Cynopterus sphinx* (B), *Rhinopoma hardwickii* (C), *R. microphyllum* (D), *Scotophillus heathii* (E), *Scotophillus kuhlii* (F), *Pipistrellus coromandra* (G), *Taphozous nudiventris* (H), *Megaderma lyra* (I) and *Hipposideros fulvus* (J).

Figure 1.2 The roosts of different bat species: *Rousettus leschenaulti* (A), *Cynopterus sphinx* (B), *Rhinopoma hardwickii* (C), *Rhinopoma microphyllum* (D), *Scotophilus heathii* (E), *Scotophilus kuhlii* (F), *Taphozous nudiventris* (G), *Megaderma lyra* (H) and *Hipposideros fulvus* (J).

Chapter 02:

Isolation and identification of guanophilic fungi

Chapter 02

Isolation and Identification of Guanophilic Fungi

Introduction

The excreta of wild birds and animals, including bats, contain medically significant fungi (Garcı´a-Hermoso *et al.*, 1997) and locations that contain large amounts of such excreta are potential sites of human infection. Bat guano is one of the most important substrates for fungi. Bat guano is a rich substrate, which combined with high humidity along with stable temperature through the year and provides optimal environmental conditions ideal for fungal growth. 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 (Martins, 1976). The composition of bat guano consuming different diets has received little attention. Poulson (1972) reported that the simple bat guano and its habitat are enough to constitute a complete ecosystem. Guanophilic fungi of bats usually serve as saprotrophs or pathogens or as transient chemo heterotrophic microorganisms (Northup *et al.*, 1997). Insectivorous bats are known to be the prime contenders as reservoirs of fungi such as *Histoplasma capsulatum*, *Coccidioides immitis*, *Cryptococcus laurentii* and *Blastomyces dermatitidis* (Yamamoto *et al.*, 1995; Garcia Hermoso *et al.*, 1997; Mattsson *et al.*, 1999; Bunnell *et al.*, 2000). The incidence of histoplasmosis being transmitted from bat droppings (guano) to humans occurs infrequently. Large colonies of bats do not normally inhabit work areas. Nevertheless, fresh bat droppings can contain the histoplasmosis fungus. Eight case of pulmonary histoplasmosis reported in young man exploring cave Cueva del eñón del Barrio Rosario, San Germán, Southwestern

Puerto Rico (Martínez, 1972). White fungus (*Geomyces destructans*) is a psychrophilic (cold-loving) fungus (Gargas *et al*., 2009) that cause white nose syndrome in bats, fungus grows on the face, ears, or wings of affected bats. Hyphae of fungi penetrate in membranes and tissues lead to severe damage (Meteyer *et al*., 2009). White nose syndrome bat either they have no fat reserve or very little which are essential for survival during hibernation (Blehert *et al*., 2009). *Geomyces destructans* is closely related to *G. pannorum*, which causes skin infections in humans (Gianni *et al*., 2003).

Fungi play an important role on the decomposing of different substrates, influencing energy flow through the subterranean food. Fungi perform duties as food resource to some of the cave-dwelling organisms (Gunn, 2004; Culver and White 2005; Estrada-Bárcenas *et al*., 2010) and help in the controlling population of other invertebrates by entomopathogenic organisms (Gunde-Cimerman *et al*., 1998; Yoder *et al*., 2009). Among the Fungi more frequently found in the subterranean ecosystems are some human opportunistic pathogens (Biswas *et al*., 2013; Resende-Stoianoff *et al.,* 2012, Taylor *et al*., 2013; Vanderwolf *et al*., 2013).The genera *Aspergillus, Paecilomyces, Purpureocillium, Fusarium, Mucor and Trichoderma* and are filamentous fungi commonly isolated from caves which also reported to be infectious to humans (Armstrong, 1993; Pfaller and Diekema, 2004; Trabulsi and Alterthum 2004).

Uttar Pradesh is known for its rich culture and tradition. There are different places such as Agra, Jhansi, Lucknow, Allahabad, Banda, Chitrakoot and Meerut are historical cities famous for their monuments. Ayodhya, Allahabad, Mathura, Vrindavan, Gokul and Varanasi are holy cities for Hindus, famous for their temples and Kushinagar and Sarnath are important Buddhist places among the main four

pilgrimage sites related to the life of Gautama Buddha. Taj Mahal, one of the Seven Wonders of the World in Agra is also located in Uttar Pradesh. Most of the bats are residing in historical monuments, fort and temple. Structures used by bats include caves, rock crevices, and diverse man made structures, although more than half of the worldwide bat fauna use plants for roosting (Kunz and Lumsden, 2003), old and dead trees are richer in cavities suitable for roosting, they may be particularly important for bats (Hutson *et al*., 2001; Parsons *et al*., 2003).

Rhinopoma hardwickii (Gray, 1831) is found in arid and semi-arid habitats, including abandoned buildings, caves, dry scrub, rocky areas, deserted mountain and wells (Rahman *et al*., 2015). In the hot summer, these bats roost in nooks, cracks and Sinha (1981) reported that *T. nudiventris* was found in ruined houses and old mosque large rocks (Benda *et al*., 2004). *Rhinopoma microphyllum* is a xerophilous species inhabiting caves, ruins, mosuque, temple and old houses (Duane and Mazin, 1996) and they also occupied the nests of swifts (Prakash, 1961). They share their roost with *Rousettus leschenaulti* and *Hipposideros speoris*, and the colony size is generally restricted to a few individuals, although Brosset (1962) observed several hundred in the cave complexes of Ellora. Vespertilionids spend the day in roosts including caves, rock crevices, tree hollows, under bark, in the foliage, or bird nests, they exhibit the gamut of social life styles from solitary to gregarious, forming large roost colonies often segregated by sex and breeding condition (Nowak, 1994).

Historical monuments, fort and temple provide unique environmental characteristics that may favour the development of many microorganisms such as fungi, bacteria etc. The most distinctive features for growth of fungi are the absence of light, high humidity and the almost constancy in temperature provided by the monuments, forts, temple and abandoned building. Bats are associated with a few

diseases that affect people. Apparently there is little information available on guanophilic fungi of Indian bats. Therefore, this study was conducted to isolate and characterize the guanophilic fungi of bats and their pathogenic and ecological role in the ecosystem.

Materials and methods

Sample Collection

Guano samples of eight insectivorous such as *Rhinopoma hardwickii*, *R. microphyllum*, *Scotophilus heathii*, *S. kuhlii*, *Pipistrellus coromandra*, *Megaderma lyra*, *H. fulvus* and *Taphozous nudiventris* and two frugivorous bats such as *Rousettus leschenaulti* and *Cynopterus sphinx* were collected for this study. A fresh polythene sheet (3 x 2 m) was spread on the floor beneath the bat roosts at early morning when bats were returning to their roost and the bat droppings were aseptically collected in sterile vials using forceps. Guano samples were stored in deep freezer (-20ºC) for further analysis.

Fungal Isolation

Isolation of guanophilic fungus was carried out by suspending 01 g of guano in 09 ml of sterile water to make 10 ml stock suspension. From the stock, 1 ml of sample was taken and diluted to 10^{-1} and similar step was repeated up to 10^{-5}. Streptomycin sulphate and tetracycline hydrochloride (8µg/l) were mixed with the Potato Dextrose Agar media (Hi-Media) as antibacterial agent for fungal culture. The fungal samples were inoculated in the culture plates and incubated at 28 ºC for 7-9 days. The 7-9 day-old was extirpated and used for pure culture in PDA media at 25 °C for 7 days by following King *et al*., (1986). The species recognition was carried out

by colony morphology such as colony diameter, texture and sporulation (Thom and Raper, 1945; Raper *et al*., 1949).

Sample preparation for Light Microscopy

A small portion of mycelia of 5 to 7-day-old fungal culture was taken aseptically on glass slide with the help of sterilized needle, spread over the microscopic slide and stained with lacto phenol cotton blue and observed under the Light Microscope (Olympus CX-40, Olympus, USA). Photographs of culture plates and slides were taken using a digital camera (Olympus C7070WZ, Olympus, USA).

Sample preparation for Scanning Electron Microscopy

The fungal samples were carefully collected from the inoculation plates and mounted on the aluminium stubs using double side carbon adhesive tapes and kept in desiccators overnight. The stubs were sputter coated with palladium coater (JFC-1800) and the morphology of fungus was studied under Scanning Electron Microscope (JEOL JSM, 6400 LV, Japan) with an accelerating voltage between 5 and 15 KV at different magnifications.

DNA Extraction

100 mg of 10-day-old fungal mycelia were harvested and kept in absolute ethanol at -20 °C overnight. Fungal samples were washed twice with distilled water to remove the ethanol and manually ground using mortar and pestle with 50 mg of sea sand. 500 µl prewarmed (60°C) TES lysis buffer (Moller*et al*., 1992), 50µg proteinase K were added in ground samples and incubated in 60°C for 45 min. 140 µl 5 M NaCl and 64 µl 10 % CTAB were added and incubated at 65 °C for 10 min. Equal volume of chloroform: isoamylalcohol (24:1) was added and kept in deep freezer for half an

hour and centrifuged at 14000 g for 15 min. DNA was precipitated by adding 0.6 volume ice cold isopropanol and 0.1 volume 3M sodium acetate, centrifuged and washed twice with 70 % ethanol. Pellet was left for complete drying and dissolved in 100 µl HPLC water. The extracted DNA was quantified using a Nanodrop spectrophotometer (Thermo, ND-1000, USA).

Amplification of DNA

For each 50 µl reaction, a mixture of 50 ng genomic DNA, 5 ml 10 X PCR buffer (Thermo Fischer), 0.2 mM deoxynucleoside triphosphate (dNTP), 20 pmol of primerITS1F (50-CTTGGTCATTTAGAGGAAGTAA-30) and ITS4 (50-TCCTCCGCTTATTGATATGC-30) and 1 U of Taq DNA polymerase (Thermo Fischer) was prepared. The amplification was performed using an automated thermal cycler (CG Palm cycler). The cycling parameters were initial denaturation at 95 ºC for 5 min, and then 30 cycles of denaturation for 30 s at 94 °C, annealing at 56°C for 30 s, and extension at 72 °C for 1 min. The amplification was terminated by a final extension for 10 min at 72 °C. Then 5 µl of PCR product were analysed on a 1.5 % agarose gel (60 V) containing 0.5 μ g/ml ethidium bromide. Amplified PCR products were send to Chromous Biotech Pvt. Ltd. for sequencing. The ITS4 and ITS5 sequences of these isolates were compared to 56 corresponding sequences of reference fungal taxa at NCBI database using nucleotide blast in the Gen Bank.

Phylogenetic analysis

The sequences were aligned using Clustal X by following Thompson*et al.* (1994). Phylogenetic trees were constructed by MEGA 6.06 (6140226) using maximum parsimony and Bayesian methods based on internal transcribed spacer (ITS) sequences. Bootstrap values were calculated from 1000 replications.

Results

Morphological Identification

There were 56 isolates of fungus belong to 32 species observed in the guano of 10 species of bats. Among the fungal isolates species belong to the genus *Aspergillus* and *Penicillium* were common. The genus *Aspergillus* contributed 26 isolates (46.42% relative isolation frequency) and *Penicillium* 12 isolates (21.42% relative isolation frequency) followed by four isolates of *Cladosporium* (7.14% relative frequency) two isolates of *Alternaria* (3.57% relative isolation frequency), two isolates of *Mucor* (3.57% relative isolation frequency) and single isolates of *Paecilomyces*, *Absidia, Sarocladium*, *Periconia*, *Trichocomaceae*, *Malbranchea*, *Davidiella* and Yeast (1.78% relative isolation frequency).

The isolates belong to 13 genera such as *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Mucor*, *Crysosporium*, *Paecilomyces, Absidia, Sarocladium*, *Periconia*, *Trichocomyces*, *Malbranchea*, and *Davidiella*.

The genus such as *Aspergillus*, *Penicillium*, *Paecilomyces*, *Trichocomyces* and *Trichocomyces* belong to the family of Trichochomaceae while genera *Cladosporium* and *Davidiella* belong to Davidellaceae family. The remaining seven genu ssuch as *Alternaria*, *Mucor*, *Crysosporium*, *Absidia*, *Sarocladium*, *Malbranchea* and *Trichoderma* belong Pleosporaceae, Mucoraceae, Onygenaceae, Cunninghamellaceae, Incertiae sedis, Myxotrichaceae and Hypocereaceae families respectively.

There were 26 isolates of genus *Aspergillus* belongs to nine species such as *A. versicolor*, *A. flavus*, *A. oryzae*, *A. stellatus*, *A. niger*, *A. sydowii*, *A. caelatus*, *A. sclerotiorum* and *Aspergillus* sp. were isolated from the guano of bats (Table 2.1).

The colony diameter of PKM2, pkm3, pkm8, pkm9, pkm10, pkm11, Pkm12, PKM15, PKM16,PKM17, PKM18, PKM22, PKM23, PKM24, PKM25,PKY1, PKY2, VE1, VE6, VE7,VE9 and Ambedkar isolates of *Aspergillus* genus were ranges from 90 mm – 11.32 mm.

Four types of texture floccose, velvety, wooly and powdery were found in all isolates of *Aspergillus*. Among 26 isolates, 19 isolates such as PKM16, PKM25, VE7, PKY2, pkm11, PKM15, PKM23, PKM18, VE1, pkm2, VE9, pkm8, VE6, Pkm12, PKY1, pkm10, PKM17, PKM22 and Ambedkar were found floccose in texture (Fig. 2.4B, 2.4D, 2.4G, 2.4H, 2.4J – 2.4P, 2.7B – 2.7D and 2.7G – 2.7K). *Aspergillus versicolor* gr. show velvety in texture (Fig. 2.4C), two isolates of *A. scleorotium* isolates pkm9 (Fig. 2.7E), *A. sydowii* strain PKY1 were also found velvety in texture (Fig. 2.7G). Only two isolates of *A. flavus* such as PKM24 and pkm3 were found wooly in texture (Fig. 2.4F and 2.4I). Powdery texture was observed in *A. niger* (Fig. 2.7F).

Two strains of *A. versicolor* such as PKM16 and PKM25 were antique bronze, porecelain green and white (Fig. 2.4B – 2.4D). The hyphae of all isolates of *A. flavus* were septate and dichotomously branched (Fig. 2.5D – 2.5N), conidial heads were radiated, uni and biseriate (Fig. 2.6D – 2.6N). Two isolates of *A. oryzae*such as VE9 and pkm8 showed navy blue and light turtle green in coloration (Fig. 2.4P and 2.7B), The conidium of VE9 was rough and globose (Fig. 2.5O, 2.6O) while the conidia of pkm8 dichotomously branched (Fig. 2.8A, 2.9A). The conidium of *A. niger* was brown to black, very rough and globose (Fig. 2.8F). The conidiophores bore numerous black dot-like spores at the terminal end (Fig. 2.9F). The hyphae were translucent and septate. The conidial heads of *A. niger* were radiated initially and thereafter split into columns. Two isolates VE6 (Fig. 2.7C) and Pkm12 (Fig. 2.7D) of *A. stellatus* was olive grey and lavender respectively. The colony of *A. scleorotium* (isolates pkm9) was old gold in

color (Fig. 2.7F) (Table 2.1). The conidial heads were uniseriate (Fig. 2.8D) and conidiophores bore chain of conidia (Fig. 2.9D). Two colonies of *Aspergillus* sp. such as PKM22 (Fig. 2.7J), and Ambedkar (Fig. 2.7K), were white in color while PKM17 was serpentine in color (Fig2.7I).

A total 12 isolates belong to nine species of *Penicillium* such *P. citrinum*, *P. oxalicum*, *P. crustosum*, *P. polonicum*, *P. capsulatum*, *P. funiculosum*, *P. concentricum*, *P. rubidurum* and *Penicillium* sp. were isolated from the guano of bats (Table 2.1). The colony diameter ranges from 6.13 mm to 90.00 mm on maturity (Table 2.1). The texture of isolates were floccose (Fig. 2.7O, 2.10B – 2.10D), velvety (Fig. 2.7L and 2.10G), powdery (Fig. 2.10F and 2.7N) and rough (Fig. 2.7M). The conidial color of two isolates VE11 and VE4 was niagra green, except these all isolates of *Penicillium* were variable in color from pale yellow to cambridge blue (Table 2.1). A large number of whip-like conidial chains observed at the terminal end of conidiophore with spherical spores (Fig. 2.11D – 2.11E, 2.11F – 2.12G and 2.12D – 2.12E, 2.12F – 2.12G). The conidiophores consists long and oval spores at the terminal end (Fig. 2.8K – 2.8N, 2.11A – 2.11C and 2.11H), while some isolates conidiophore was branched and bore chains of conidia at the terminal end (Fig. 2.9K, 2.9N, 2.12A – 2.12C and 2.12H).

Four species of *Cladosporium* such as *C. tenuissimum* (isolates VE8), C. *cladosporoides*, *C. resinae* and *Cladosporium* sp. were isolated from the guano samples. The colony diameter varies from 41.59 mm to 9.39 mm (Table 2.1). The color of conidiophores of all four species of *Cladosporium* differed from cream to pale glaucose green (Fig. 2.10J). All four species *Cladosporium* had velvety in texture (Fig. 2.10 J – 2.10M). The conidiophores of *C. tenuissimum* isolates VE8 were tall, upright, branched variously near the apex, clustered or single (Fig. 2.11I). The conidia

(blastospores) are also dark brown in color, they are variable in shape and size (Fig. 2.12I). Some of them are ovoid and others are cylindrical, often in simple or branched acropetalous chains. *Cladosporium cladosporoides* was isolated from a roost at the river bank of Ganga, Varanasi and *C. resinae* was isolated from a monument at Sultanpur. The septate hyphae of *C. cladosporoides* had spherical conidia (Fig. 2.11J). The conidia were sparsely attached on the hyphae (Fig. 2.12J). *Cladosporium resinae* first conidium was developed in to ramoconidia with three protuberant scars and conidiophores arose laterally from vegetative hyphae (Fig. 2.11K, 2.12K). All species of *Cladosporium* were found moderate in sporulation.

The culture of the *Alternaria* colony was initially white but gradually turned into benzo brown to cinnamon drab color (Table 2.1). The colony diameter of *Alternaria* ranged from 41.59 to 32.8 mm on seventh day of culture (Table 2.1). Aged culture appeared completely greyish with aerial mycelium and distinct concentric rings was formed on the medium (Fig. 2.10N – 2.10O). Conidiophores of *A. tenuissima* strain NKG1 were short, simple or branched arising singly (Fig. 2.12M), while *Alternaria* sp. NKG2 waslong and septate (Fig. 2.12 N).

The colony of *Mucor* sp. was white, rounded (Fig. 2.10P and 2.13B) and colony diameter varied from 58.01 – 13.88 mm (Table 2.1). The hyphae were hyaline with terminal conidia (Fig. 2.120 and 2.15A). *M. indicus* isolates BBAU velvety in texture but *Mucor* sp. was wooly. Sporulation of both isolates on czapek dox agar medium was mild.

Chrysosporium tropicum was isolated from the guano samples of *R. hardwickii* collected from Jhusi fort, Allahabad. The colony of *C. tropicum* was white, wooly, rounded and attained 7.58 mm at maturity (Fig. 2.13C). The thin walled hyphae were

hyaline, branched and divided by septa. The barrel-shaped conidium was solitary, terminal and stalked (Fig. 2.14B and 2.15B).

The colony of *P. varitii* was wooly, rounded, burly wood color and attained a diameter 66.29 mm (Fig. 2.13D). The hyphae were septate, branched and bore chains of spores (Fig. 2.14C, 2.15C).

Absidia corymbifera was isolated from the guano samples of *R. hardwickii* collected at Khusrubagh fort, Allahabad and *R. microphyllum* guano from Godavari cave temple, Chitrakoot. The colony of *A. corymbifera* was white, wooly, rounded and attained 37.93 mm at maturity (Fig.2.13E). The branched conidiophores bore few numbers of conidia (Fig. 2.14D, 2.15D).

The colony of *Malbranchea* sp. was isolated from the guano samples of *R. hardwickii* collected from Khusrubagh and Allahabad. The colony of *Malbranchea* sp. was bronze colored, velvety textured and attained 14.62 mm (Fig. 2.13G). The branched and segmented hyphae bore spores (Fig. 2.14F and 2.15F).

The colony of *Trichoderma* sp. was acid green, wooly and attained 58.01 mm (Fig. 1O). The hyphae were highly branched and bore spores (Fig. 2.13H). The Yeast colony was pink, wooly, rounded, non-sporulating and attained 7.72 mm with capsule like individual Yeast (Fig. 2.14G and 2.15G).

The isolate of *S. implicatum* (isolate pkm4), *Periconia* sp. (VE2), *Trichocomaceae* sp. (PKM21) and *Davidiella* sp. (PKM20) were isolated from bat guano. The colony diameter ranged from 20.6 to 9.85 mm on maturity (Table 2.1). *Trichocomaceae* sp. PKM21 and *Davidiella* sp. PKM20 were floccose in texture (Fig. 2.13J and 2.13K). *S. implicatum* (isolate pkm4) was velvety while *Periconia* sp. VE2

was wooly in texture (Fig. 2.13F and 2.13I). The isolates such as pkm4, VE2 and PKM21 were found very little in sporulation while PKM20 was moderate in sporulation.

The Yeast colony was pink, wooly, rounded, non-sporulating and attained 7.72 mm (Fig. 2.13L).In addition, sterile mycelia were isolated from the guano samples of *R. hardwickii* collected from Atla mosque, Jaunpur, Jhunsi fort, Allahabad and Godavari cave, Chitrakoot. The colonies of sterile mycelia were white, cottony, rounded and non-sporulating.

Molecular identification

The fungal isolates of insectivorous bats were grouped as Eurotiomycetes, Sordariomycetes, Ascomycetes and Dothideomycetes through a phylogenetic tree (Fig. 2.2). The group Eurotiomycetes includes genera *Aspergillus*, *Penicillium* and *Trichocomacea* kept in clade I. The fungal group Sordariomycetes consists genus *Sarcocladium* in Clade II. *Pericornia* falls under clade III which comes under class Ascomycetes. A well-supported clade IV formed with boot strap value 100% which include class Dothideomycetes genus *Alternaria*.

The isolates of *A. flavus* such as pkm2, pkm11, PKM 23 and PKM24, *A. caelatus* isolate pkm10 as well as the isolates of *A. oryzae* such as pkm8 and VE9 were grouped into clade I with 95% bootstrap support (Fig. 2.2). *A. stellatus* isolate Pkm12, *A. sydowii* strain PKY1, *A. versicolor* strain PKM25 and *Trichocomaceae* sp. PKM21 were placed in the same clade I with 100% supported boot strap value (Fig. 2.2) while *P. oxalicum* isolate pkm7, *P. crustosum* strain PKM19, *P. polonicum* isolate Pkm13, *P. capsulatum* isolate pkm14, *P. rubidurum* isolate pkm5 were also placed into the same with boot strap value 41%.

Three clades were formed from the sequences of fungus isolated from the guano of frugivorous bats such as clade I (Class Eurotiomycetes), clade II (Class Mucormycotina) and clade III (Class Dothideomycetes) (Fig. 2.3).

Four isolates of *A. flavus* such as PKM15, PKM18, PKY2 and VE1, single isolates of *A. versicolor* strain PKM18 and *Aspergillus* sp. Ambedkar were grouped in clade I with 100% supported boot strap value.

One isolates of *M. indicus* isolate*s BBAU* were placed in Clade II (Fig. 2.3). Two species of *Cladosporium* sp. VE3 and *C. tenuissimum* isolate VE8 were placed in well supported clade III with boot strap value 91%.

Discussion

The diversity of guanophilic fungi of two frugivorous and eight insectivorous bats was accessed by morphological and molecular methods. A total of 56 isolates of fungi were isolated and identified from different species of bats. Out of them 18 isolates were isolated from the guano of frugivorous bats while 38 were isolated from the guano of insectivorous bats. A maximum diversity of guanophilic fungi (15 isolates) was observed in the guano of *R. hardwickii* while least was observed in *R. microphyllum* and *P. coromendra* single isolates. Out of these, maximum diversity of the genus *Aspergillus* followed by *Penicillium* were recorded in the present study. Whereas in case of *M. lyra* nine isolates were observed and all of them reported to be of the same genus *Aspergillus*. Sum of six isolates each from the guano of *H. fulvus* and *S. kuhlii* were isolated.

Six new strains of *A. flavus* such as PKM24, PKY2, PKM15, PKM23, PKM18 and VE1 were reported for the first time from the guano of bats. *Aspergillus flavus*

causes chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis. It also causes otitis, cutaneous aspergillosis, pulmonary and systemic infections in immunocompromised patients.

Another two such similar strains of *A. versicolor* such as PKM25 and PKM16 were found in the guano of *C. sphinix* and *P. corromendra* bats which were not yet deciphered. *Aspergillus versicolor* found in the guano was widely isolated from soil, indoor environments (Shelton *et al*., 2002; Engelhart *et al*., 2002; Amend *et al*., 2010; Anderson *et al*., 2011), various foods and hyper saline water (Kis-Papo *et al*., 2003; Mbata, 2008) and also associated with many health issues of humans and animals (Perri*et al*., 2005; Baddley *et al*., 2009; Edmondson *et al*., 2009; Moreno and Arenas, 2010). *Aspergillus versicolor* and *A. niger* were known to cause severe lung problems (Aspergillosis) to human if inhaled in sufficient amount (Ajay *et al*., 2011). The mycotoxin produced by *A. niger* causes several ailments of liver, kidney, nervous system, muscles, skin, respiratory organs, digestive tract and genital organs in human (Durakovic *et al*., 1989; Rai and Mehrotra, 2005). The cosmopolitan fungus *A. niger* produces ochratoxin A, fumonisin B2 and aflatoxin in stored commodities (Schuster *et al*., 2002, Noonimabe *et al*., 2009, Al-Abdalall, 2009).

Penicillium is a diverse fungal genus with 354 accepted species today (Visagie *et al*., 2014), which was second most abundant fungus found in the studied bat guano. The fungi of the genus *Penicillium* are economically diverse with a wide array of medical, industrial and environmental importance. The occurrence of *P. funiculosum* in bat guano attributes that the insectivorous bat *R. hardwickii* consumes the insects or insect pests which are reservoir of *P. funiculosum*. The filamentous fungus *P. funiculosum* involves in cellulose production (Roberto *et al*. 2013) to secrete a balanced cellulasic system (Rao *et al*., 1988; Castro *et al*., 2010). The existence of *P. funiculosum* in the

plant parts and the insects around the plants was well established (Lim and Rohrbach, 1980).

New strains of *Penicillium crustosum* strain PKM19 were deciphered first time from the guano of *T. nudiventris* bat guano. *P. crustosum* convert testosterone to 5α-dihydrotestosterone (DHT) (Flores *et al.*, 2003), for this 5 α -Reductase enzyme is responsible. This enzyme is also present in animal and human androgen-dependent tissues as well as in prostate and seminal vesicles. The increase of the conversion of testosterone to DHT in prostate glands has been related to some illnesses such as benign prostate hyperplasia (Carson and Rittmaster, 2003) and prostate cancer (Van Der Sluis *et al.*, 2011).

Penicillium oxalicum and *P. citrinum* were isolated from the guano of *R. leischnaulti*, *M. lyra*and *H. fulvus* roosting in old building and historical monuments. Multiple allergens have been characterized from *P. chrysogenum* (formerly *P. notatum*), *P. citrinum*, *P. brevicompactum*, and *P. oxalicum* that could play a role in inducing some of the symptoms associated with exposure to the organism in sick buildings, many of which are proteases and are summarized in the following reviews (Shen *et al.*, 1999; Kurup *et al.*, 2000; Bush and Portnoy, 2001; Kurup, 2003). Recent studies have identified *P. citrinum* as a possible candidate in the etiology of sago hemolytic disease (Atagazli*et al.*, 2010), is an acute hemolytic syndrome affecting rural Papua New Guineans who depend on the starch of Metroxylon sagu as a staple carbohydrate.

Penicillium capsulatum were found in the guano of *H. fulvus*, it is well known to produce extracellular enzymes, which are mainly used in paper production (Paice and Jurasek, 1984) but recently a case report of pulmonary fungus ball is caused by *P.*

capsulatum in a 56-year-old Chinese female gardener patient with type-2 diabetes (Chen *et al*., 2013).

Absidia corymbifera is opportunistic mycoses by causing zygomycosis in immunocompetent hosts (Hagensee *et al*., 1994; Ribes *et al*., 2000) and most commonly reported as an animal pathogen and causes mycotic abortion in cows (Knudtson and Kirbride, 1992).

Cladosporium cladosporioides listed as occasional agent of phaeohyphomycosis and the infections of *C. cladosporioides* are extremely rare (Matsumoto *et al*., 1994). *Cladosporium resinae* found in guano of *R. hardwickii* was widely observed in soil and actively decomposes hydrocarbons (Ahearn and Meyers, 1972). *Paecilomyces varitii* observed in the guano of *R. hardwickii* was reported in earlier studies in the substrate including pasteurized food, soil, indoor air and wood (Samson, 1974; Pitt and Hocking, 2009).

Chrysosporium tropicum is a potent keratinophilic fungus it decomposes the most abundant and highly stable animal protein keratin (Avasan *et al*., 2011). As bats feeds on Orthopteran, Dictyopteran, Lepidopteran, Hymanopteran, Coleopteran and Dipteran insects, the occurrence of *C. tropicum* in its guano is ecologically important for decomposing the insect keratin.

Paecilomyces is listed among the emerging causative agents of opportunistic mycoses in immune compromised hosts, cutaneous or catheter related associated with almost any organ or system of the human body (Salle *et al*., 2005) and it causes hyalohyphomycosis (Ajello, 1986).

Mucor isolated from the guano of bats causes opportunistic infections zygomycosis (Larone, 1995; Stewart *et al*., 1999). *Trichoderma* sp. is most promising and an effective biocontrol agent for vegetable diseases and an antagonist controlling wide range of microbes and their mechanism of mycoparasitism involves nutrient competition, hyperparasitism and antibiosis (Weinding, 1934).

Thus, the results of current study reveal the diversity of guanophilic fungi of insectivorous bats and their role on ecosystem and human health.

Table 2.1 C**olony characteristics of guanophilic fungi isolated from the guano of ten bats species.**

Fungal Species	**Colony Diameter (mm)**	**Colony characters**			**Molecular Characterization**	
		Surface colour	Texture	Sporulation	Absorbance of DNA (260/280 nm)	Amount of DNA (ng/µl)
A. versicolor strain PKM16	16.04	Porcelain green	Floccose	Highly	1.74	769.8
A. versicolar gr.	14.4	Antique bronze	Velvety	Moderate	-	-
A. versicolor strain PKM25	17.36	White	Floccose	Highly	1.8	3069.1
A. flavus	13.84	White green	Floccose	Highly	-	-
A. flavus strain PKM24	18.11	Chamois	Woolly	Moderate	1.74	755
A. flavus isolate VE7	13.99	Cobalt melon	Floccose	Moderate	1.83	609.2
A. flavus strain PKY2	14.90	Olive lake	Floccose	Highly	1.87	3061.5
A. flavus isolate pkm3	37.89	Pallid purplish gray	Woolly	Highly	1.83	1371.2
A. flavus isolate pkm11	30.85	Lime green	Floccose	Highly	1.77	741.6
A. flavus strain PKM15	25.36	Greyish lavender	Floccose	Moderate	1.87	667.1

A. flavus strain PKM23	90	Mustard yellow	Floccose	Highly	1.88	1477.3
A. flavus strain PKM18	31.65	Storm grey Green with white	Floccose	Moderate	1.74	789.5
A. flavus strain VE1	20.08	Dark green	Floccose	Moderate	1.78	1004.4
A. flavus isolate pkm2	11.32	Yellow	Floccose	Highly	1.85	2422.5
A. oryzae isolate VE9	23.68	Navy blue	Floccose	Moderate	1.72	37.6
A. oryzae isolate pkm8	26.59	Light turtle green	Floccose	Moderate	1.76	699.5
A. stellatus isolate VE6	16.12	Olive grey	Floccose	Highly	1.86	2194.1
A. stellatus isolate Pkm12	20.82	Lavender	Floccose	Moderate	1.68	735.7
A. sclerotiorum isolate pkm9	28.7	Old gold	Velvety	Moderate	1.86	2194.1
A. niger	50.08	Black with White margin	Powdery	Highly	1.87	1302.4
A. sydowii strain PKY1	23.1	Pale voilet grey	Velvety	Moderate	1.94	2590.6

A. caelatus isolate pkm10	40.3	Yellow	Floccose	Highly	1.78	2512.5
Aspergillus sp. PKM17	32.33	Serpentine green	Floccose	Highly	1.87	667.1
Aspergillus sp. PKM22	20.26	White	Floccose	Highly	1.53	1106.1
Aspergillus sp. Ambedkar	27.24	White	Floccose	Highly	1.72	3708.6
Penicillium citrinum isolate pkm1	13.57	Pale yellow	Velvety	Very little	1.88	847.7
P. citrinum isolate pkm6	16.35	Green	Rough	Very little	1.95	2406.5
P. oxalicum isolate VE11	30.2	Niagra green	Powdery	Highly	1.99	116.2
P. oxalicum isolate pkm7	17.52	Blue with white	Floccose	Moderate	1.83	820.1
P. crustosum strain PKM19	21.22	Columbia blue	Floccose	Moderate	1.79	798.5
P. polonicum isolate Pkm13	10.6	Moss green	Floccose	Very little	1.84	2541.1
P. capsulatum isolate pkm14	90	Yellow	Floccose	Highly	1.91	1208.5

P. funiculosum	32.35	White with brown appearance	Cottony	Moderate	-	-
P. concentricum isolate VE4	18.02	Niagra green	Powdery	Highly	1.84	1213.2
P. rubidurum isolate pkm5	28.26	Dawn gray	Velvety	Very little	1.84	1146
Penicillium sp.	28.42	Dark moss green	Velvety	Moderate	-	-
Penicillium sp.	6.13	Cambridge blue	Velvety	Moderate	-	-
C. tenuissimum isolate VE8	15.6	Cream		Moderate	1.86	1044.5
C. cladosporiodes	9.39	Bronze yellow	Velvety	Moderate	-	-
C. resinae	16.43	Dark brown	Velvety	Moderate	-	-
Cladosporium sp.VE3	13.8	Pale glaucose green	Velvety	Moderate	1.87	1349
A. tenuissima strain NKG1	41.59	Benzo brown	Woolly	Mild	1.87	1832.1

Alternaria sp. NKG2	32.8	Cinnamon drab	Woolly	Mild	1.72	903.2
M. indicus isolate BBAU	13.88	White	Velvety	Very little	1.31	4871.1
Mucor sp.	58.01	White	Woolly	Non sporulating	-	-
C. tropicum	7.58	White cream	Woolly	Moderate	-	-
P. varitii	66.29	Burly wood	Suede-like	Highly	-	-
A. corymbifera	58.01	White	Woolly	Non sporulating	-	-
S. implicatum isolate pkm4	9.85	Light pinkish liac	Velvety	Very little	1.86	1651.3
Malbranchea sp.	14.62	Bronze	Velvety	Moderate	-	-
Trichoderma sp.	58.01	Acid green	Woolly	Very little	-	-
Periconia sp. VE2	10	White	Woolly	Very little	1.86	1651.3
Trichocomaceae sp. PKM21	17.6	Goblin blue	Floccose	Highly	1.94	3155.7

Davidiella sp. PKM20	20.6	Green	Floccose	Moderate	1.89	188.9
Yeast	7.72	Pink	Watery	Non sporulating	-	-

Table 2.2 Morphological identification, Gen Bank accession numbers and most resemblance BLAST search for sequences of the fungal isolates from the guano of six insectivores bat.

				BLAST Search for sequence		
Fungal species	**Fungal strain**	**Fungal isolates**	**Gene Bank accession No.**	**Reference accession No.**	**Query Coverage (%)**	**Max Identity (%)**
Aspergillus versicolor	PKM25	-	KR611591	JQ409275	90	98
A. flavus	PKM24	-	KR611590	KJ863514	99	99
A. flavus	-	pkm11	KP418791	KJ863514	93	98
A. flavus	PKM23	-	KR611589	KJ863514	98	99
A. flavus	-	pkm2	KP418782	KJ175418	86	99
A. oryzae	-	VE9	KT002572	KU978916	100	100
A. oryzae	-	pkm8	KP418788	KP172534	87	99
A. stellatus	-	Pkm12	KP418792	KU870657	92	99
A. sydowii	PKY1	-	KR611596	FJ011539	99	99

A. caelatus	-	pkm10	KP418790	JQ676205	93	98
Aspergillus sp.	PKM17	-	KR611583	HQ832844	99	99
Aspergillus sp.	PKM22	-	KR611588	KJ863514	98	93
Penicillium oxalicum		pkm7	KP418787	JF731233	98	100
P. crustosum	PKM19	-	KR611585	KJ572261	100	99
P. polonicum	-	Pkm13	KP418793	JF731269	85	92
P. capsulatum	-	pkm14	KP418794	JX841240	97	99
P. rubidurum	-	pkm5	KP418785	HQ608058	99	99
Alternaria tenuissima	NKG1	-	KR611592	JN038456	93	98
Alternaria sp.	NKG2	-	KR611593	JN038452	95	99
Sarocladium implicatum		pkm4	KP418784	KJ524675	99	99
Periconia sp.	VE2	-	KR611595	JX984780	73	96
Trichocomaceae sp.	PKM21	-	KR611587	KJ461390	91	99

Table 2.3 Morphological identification, Gen Bank accession numbers and most resemblance BLAST search for sequences of the fungal isolates from the guano of two frugivorous bats.

				BLAST Search for sequence		
Fungal species	**Fungal strain**	**Fungal isolates**	**Gene Bank accession No.**	**Reference accession No.**	**Query Coverage (%)**	**Max Identity (%)**
Aspergillus versicolor	PKM16	-	KR611582	FJ011545	99	99
A. flavus	-	VE7	KT002570	KU296261	100	99
A. flavus	PKY2		KR611597	FJ011545	99	99
A. flavus	-	pkm3	KP418783	KF738806	75	98
A. flavus	PKM15	-	KR611581	KJ934702	99	100
A. flavus	PKM18	-	KR611584	FJ011539	99	100
A. flavus	VE1	-	KR611594	KJ461401	99	99
A. stellatus	-	VE6	KT002569	KJ863518.	100	100
A. sclerotiorum	-	pkm9	KP418789	FR733827	97	99

Aspergillus sp. *Ambedkar*	Ambedkar	-	KP292571	KJ934702	98	99
Penicillium citrinum	-	pkm1	KP418781	LT558895	99	99
P. citrinum	-	pkm6	KP418786	KT844552	98	99
P. oxalicum	-	VE11	KT002573	HQ871989	100	100
P. concentricum	-	VE4	KT002567	EU551180	99	99
Cladosporium tenuissimum	-	VE8	KT002571	KT186159	100	100
Cladosporium sp.	VE3	-	KT002566	LN834368	100	99
Mucor indicus	-	BBAU	KT002574	KU571498	100	98
Davidiella sp.	PKM20	-	KR611586	HG936499	98	95

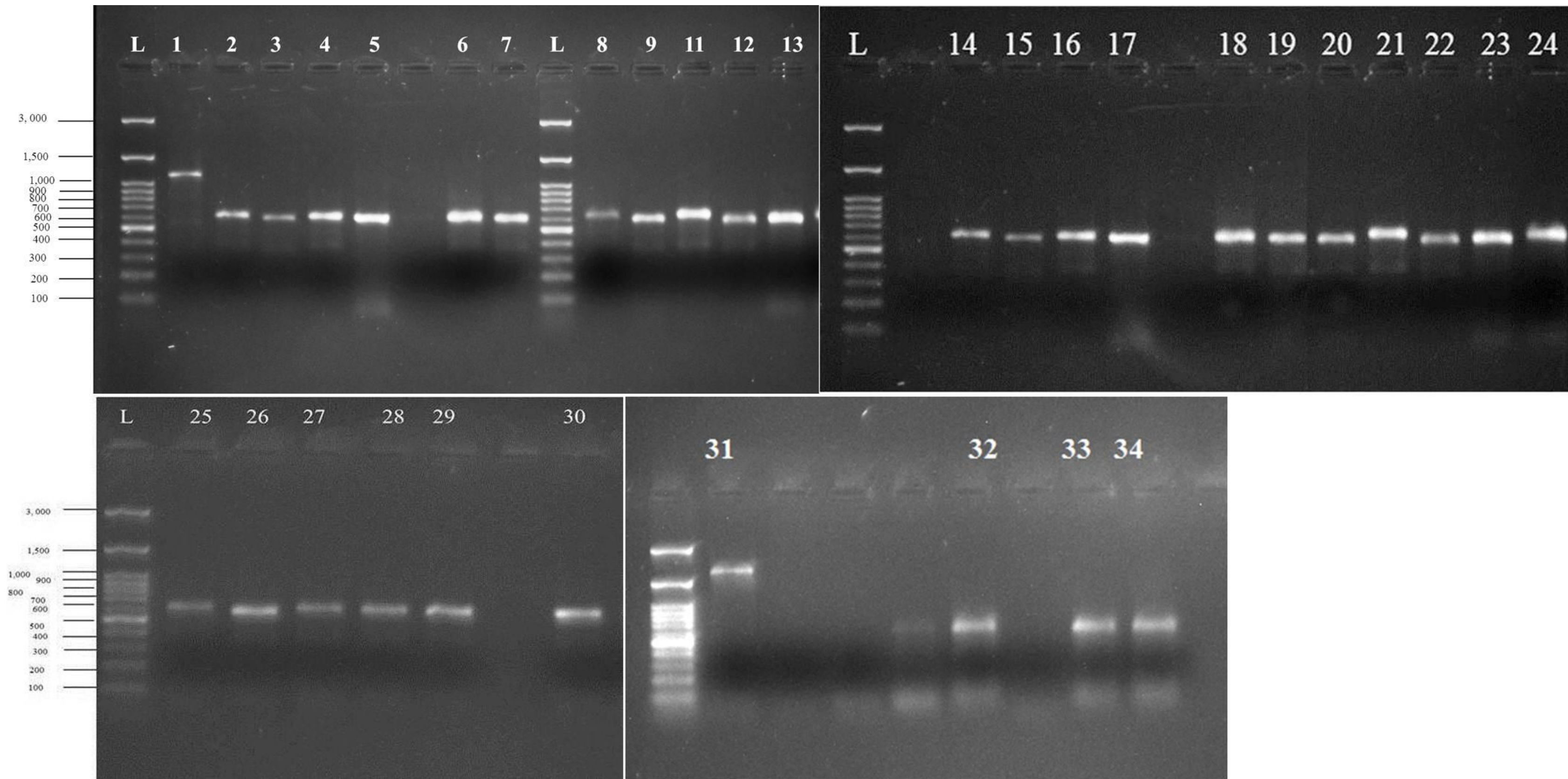


Fig. 2.1 Amplification products obtained from gDNA isolated from guano of bats amplified with ITS4 and ITS5 primers. Lane L: ladder, lane 1: *Mucor* sp., lane: 2 – 21 *Aspergillus* sp., lane 22 – 26 *Penicellium* sp., lane 27 – 28 *Cladosporium* sp., lane 29 – 30: *Alternaria* sp., lane 31: *Sarcocladium* sp., lane 32: *Pericornia* sp., lane 33: *Trichocomaceae* sp.

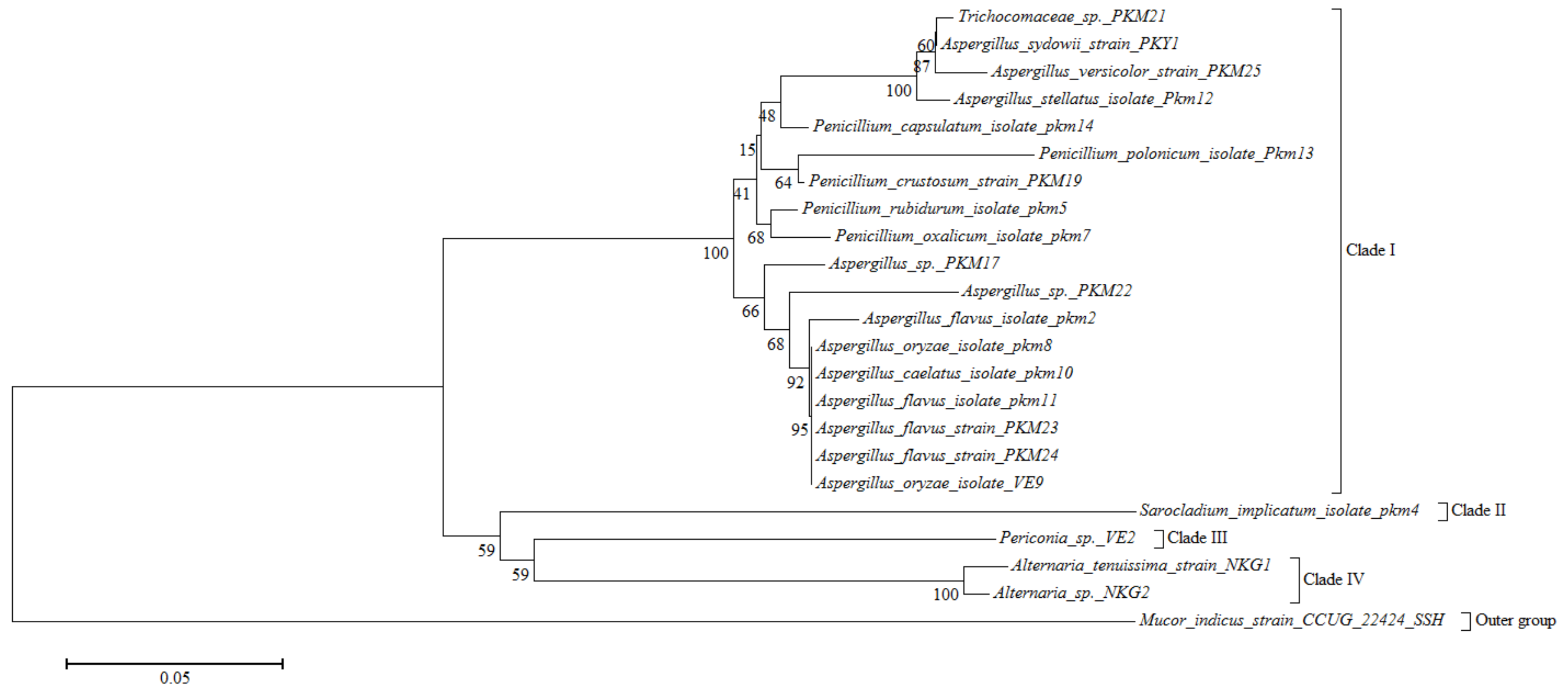


Figure 2.2 Phylogenetic analysis of fungi isolated from the guano of insecivores bat was performed based on ITS1 and ITS5 gene data using neighbor joining (NJ) with bootstrap value 1000 replications. Mucor indicus strain CCUG22424SSH were included as outgroups in ITS phylogeny.

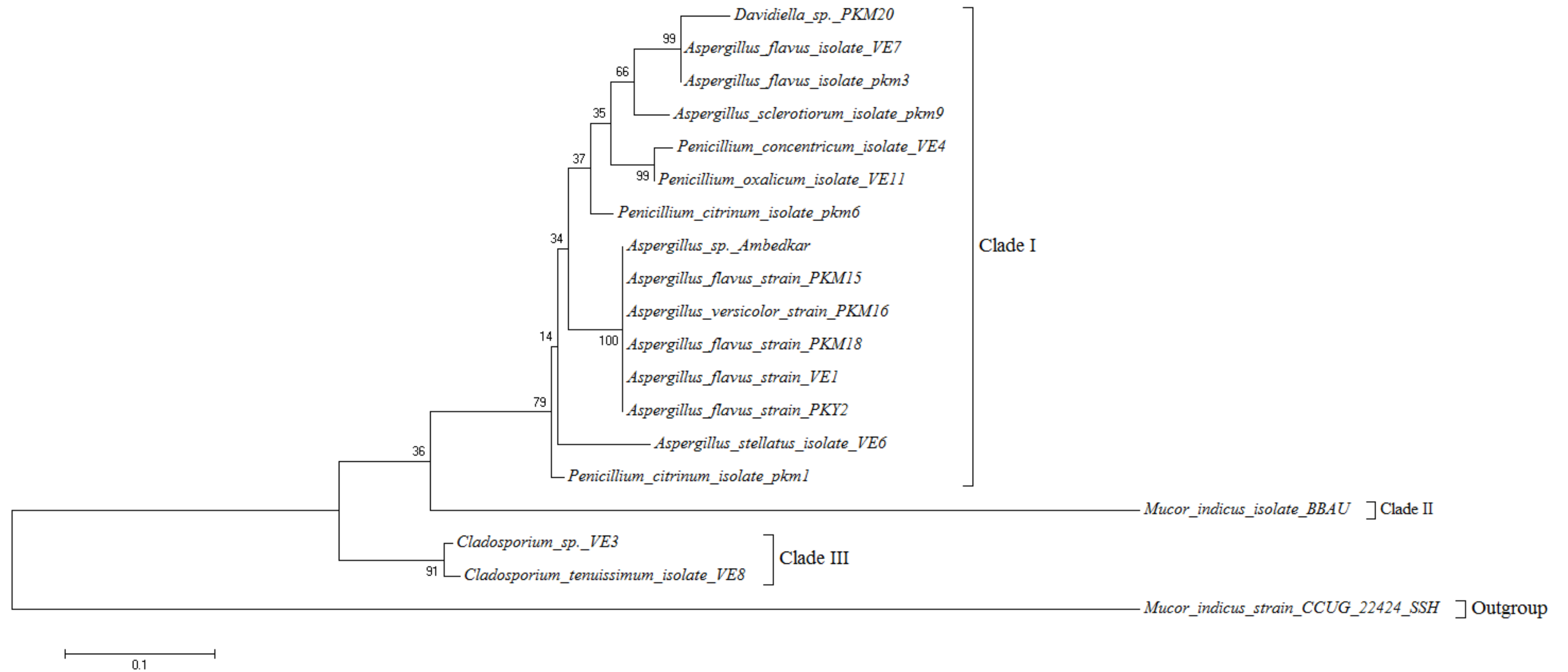


Figure 2.3 Phylogenetic analysis of fungi isolated from the guano of frugivores bat was performed based on ITS1 and ITS5 gene data using neighbour joining (NJ) with bootstrap value 1000 replications. *Mucor indicus* strain CCUG22424SSH were included as out group in ITS phylogeny.

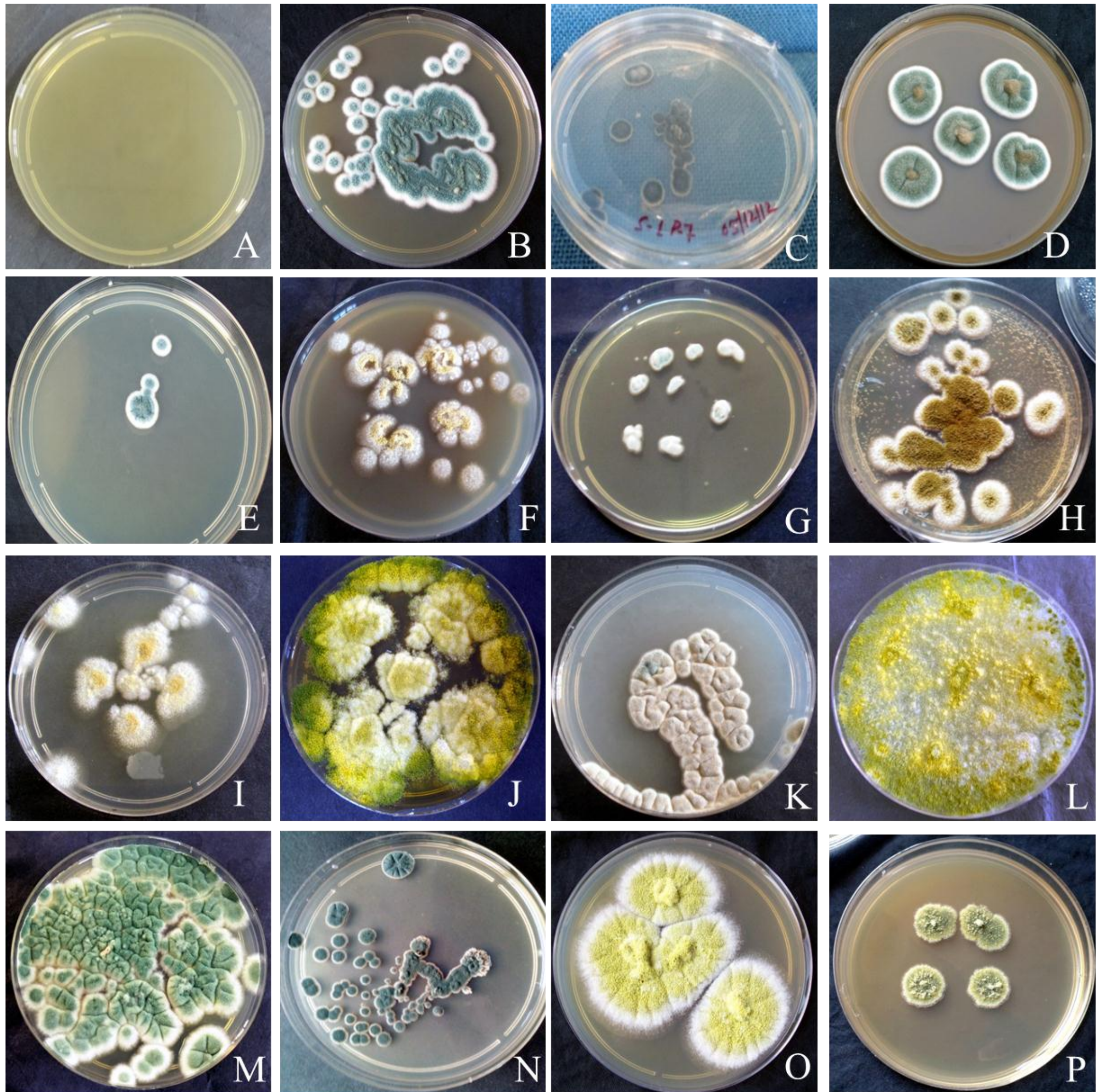


Figure 2.4 Colony morphology of fungi isolated from the guano of bats (A) Control, (B) *Aspergillus versicolor* strain PKM16, (C) *A. versicolar* gr., (D) *Aspergillus versicolor* strain PKM25, (E) *A. flavus*, (F) *Aspergillus flavus* strain PKM24, (G) *Aspergillus flavus* isolate VE7, (H) *Aspergillus flavus* strain PKY2, (I) *Aspergillus flavus* isolate pkm3, (J) *Aspergillus flavus* isolate pkm11, (K) *Aspergillus flavus* strain PKM15, (L) *Aspergillus flavus* strain PKM23, (M) *Aspergillus flavus* strain PKM18, (N) *Aspergillus flavus* strain VE1, (O) *Aspergillus flavus* isolate pkm2 and (P) *Aspergillus oryzae* isolate VE9.

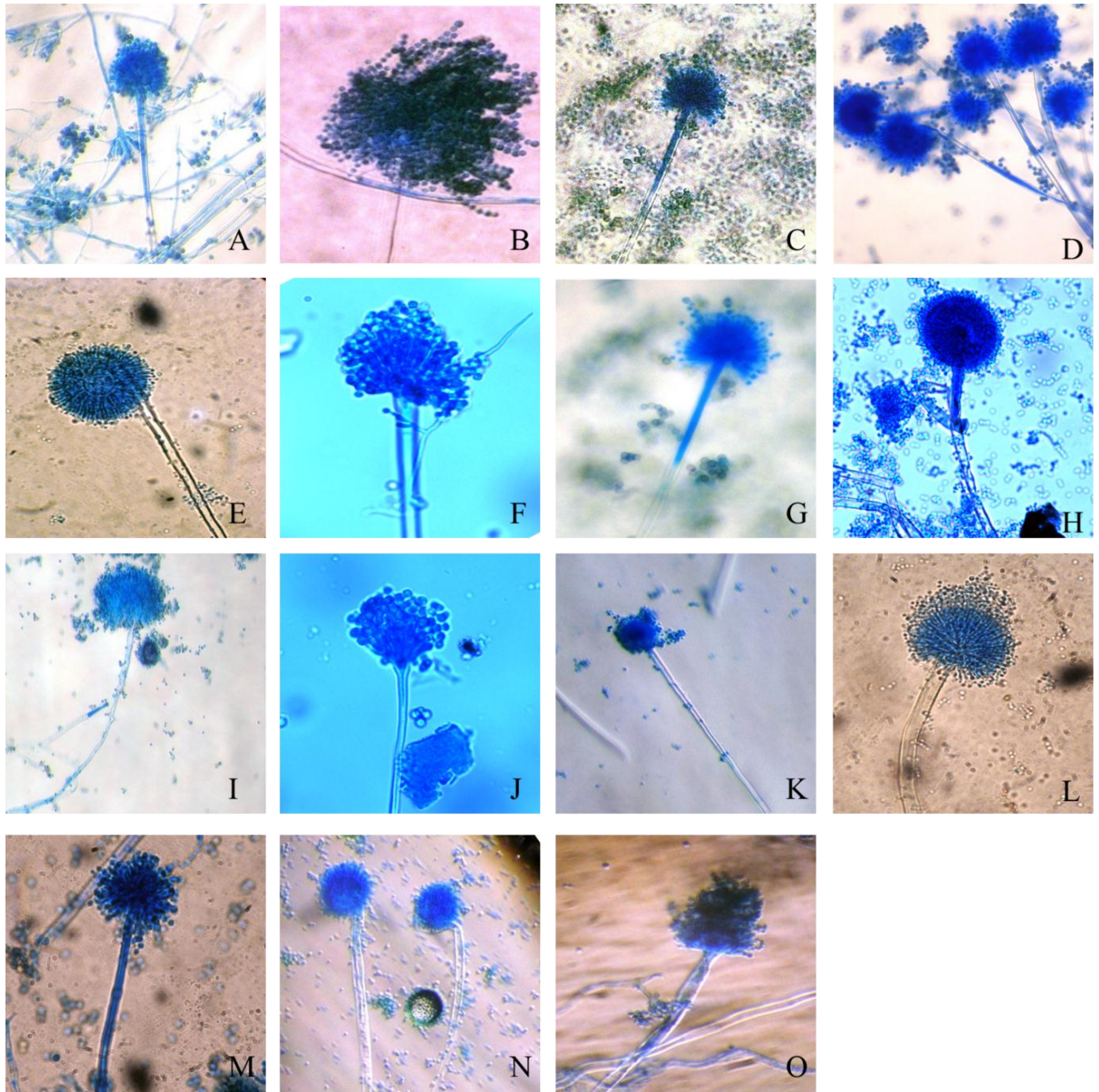


Figure 2.5 Light micrographs of fungi isolated from the guano of (A) *A. versicolor* strain PKM16, (B) *A. versicolar* gr., (C) *A. versicolor* strain PKM25, (D) *A. flavus*, (E) *A. flavus* strain PKM24, (F) *A. flavus* isolate VE7, (G) *A. flavus* strain PKY2, (H) *A. flavus* isolate pkm3, (I) *A. flavus* isolate pkm11, (J) *A. flavus* strain PKM15, (K) *A. flavus* strain PKM23, (L) *A. flavus* strain PKM18, (M) *A. flavus* strain VE1, (N) *A. flavus* isolate pkm2 and (O) *A. oryzae* isolate VE9.

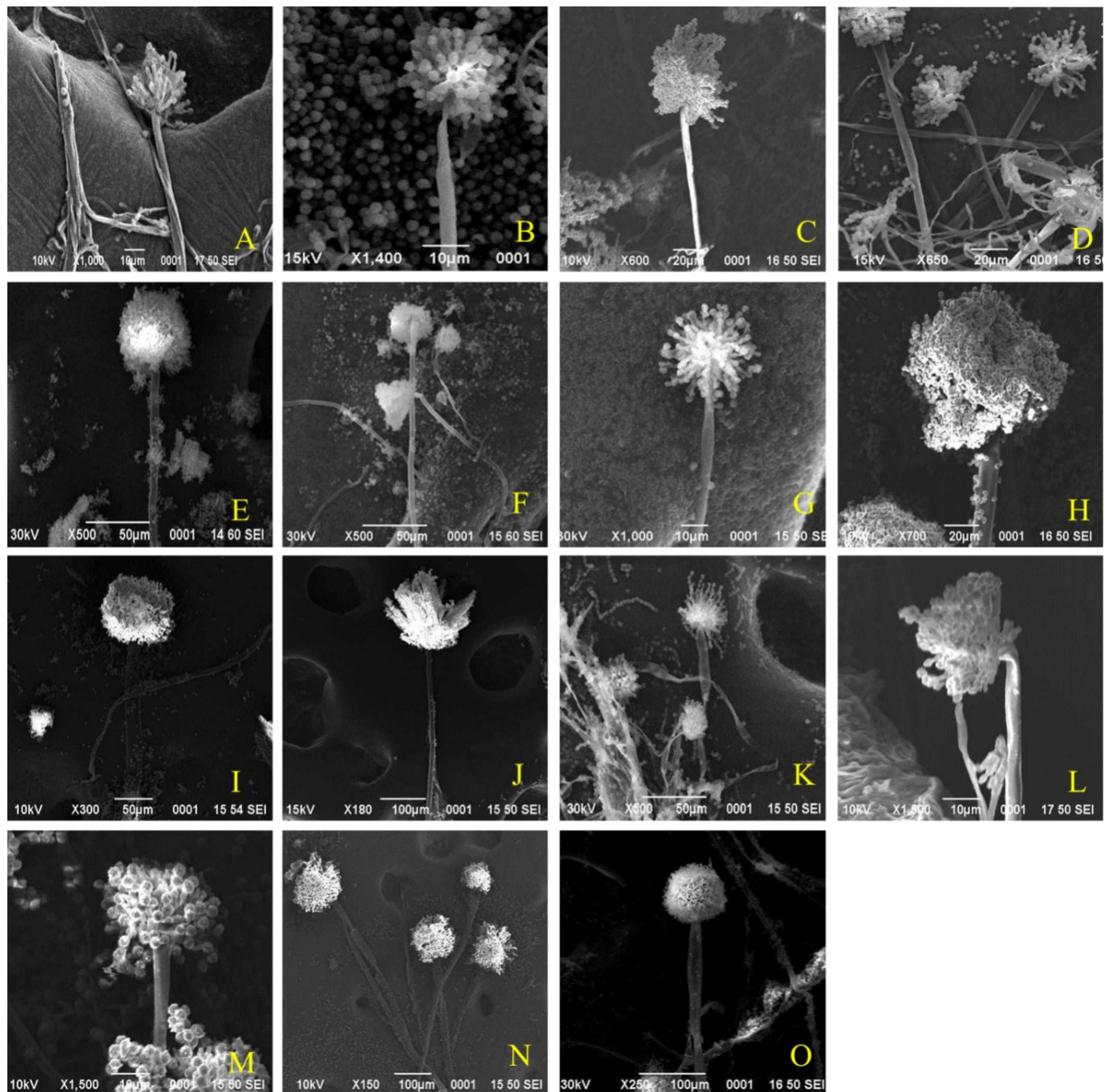


Figure 2.6 Scanning Electron micrographs of fungi isolated from the guano (A) *Aspergillus versicolor* strain PKM16, (B) *A. versicolar* gr., (C) *A. versicolor* strain PKM25, (D) *A. flavus*, (E) *A. flavus* strain PKM24, (F) *A. flavus* isolate VE7, (G) *A. flavus* strain PKY2, (H) *A. flavus* isolate pkm3, (I) *A. flavus* isolate pkm11, (J) *A. flavus* strain PKM15, (K) *A. flavus* strain PKM23, (L) *A. flavus* strain PKM18, (M) *A. flavus* strainVE1, (N) *A. flavus* isolate pkm2 and (O) *A. oryzae* isolate VE9.

Figure 2.7 Colony morphology of fungi isolated from the guano of bats (A) Control, (B) *Aspergillus oryzae* isolate pkm8, (C) *A. stellatus* isolate VE6, (D) *Aspergillus stellatus* isolate Pkm12, (E) *A. sclerotiorum* isolate pkm9, (F) *A. niger*, (G) *A. sydowii* strain PKY1, (H) *Aspergillus caelatus* isolate pkm10, (I) *Aspergillus* sp. PKM17, (J) *Aspergillus* sp. PKM22, (K) *Aspergillus* sp. Ambedkar, (L) *Penicillium citrinum* isolate *pkm1*, (M) *Penicillium citrinum* isolate *pkm6*, (N) *Penicillium oxalicum* isolate VE11 and (O) *Penicillium oxalicum* isolate pkm7.

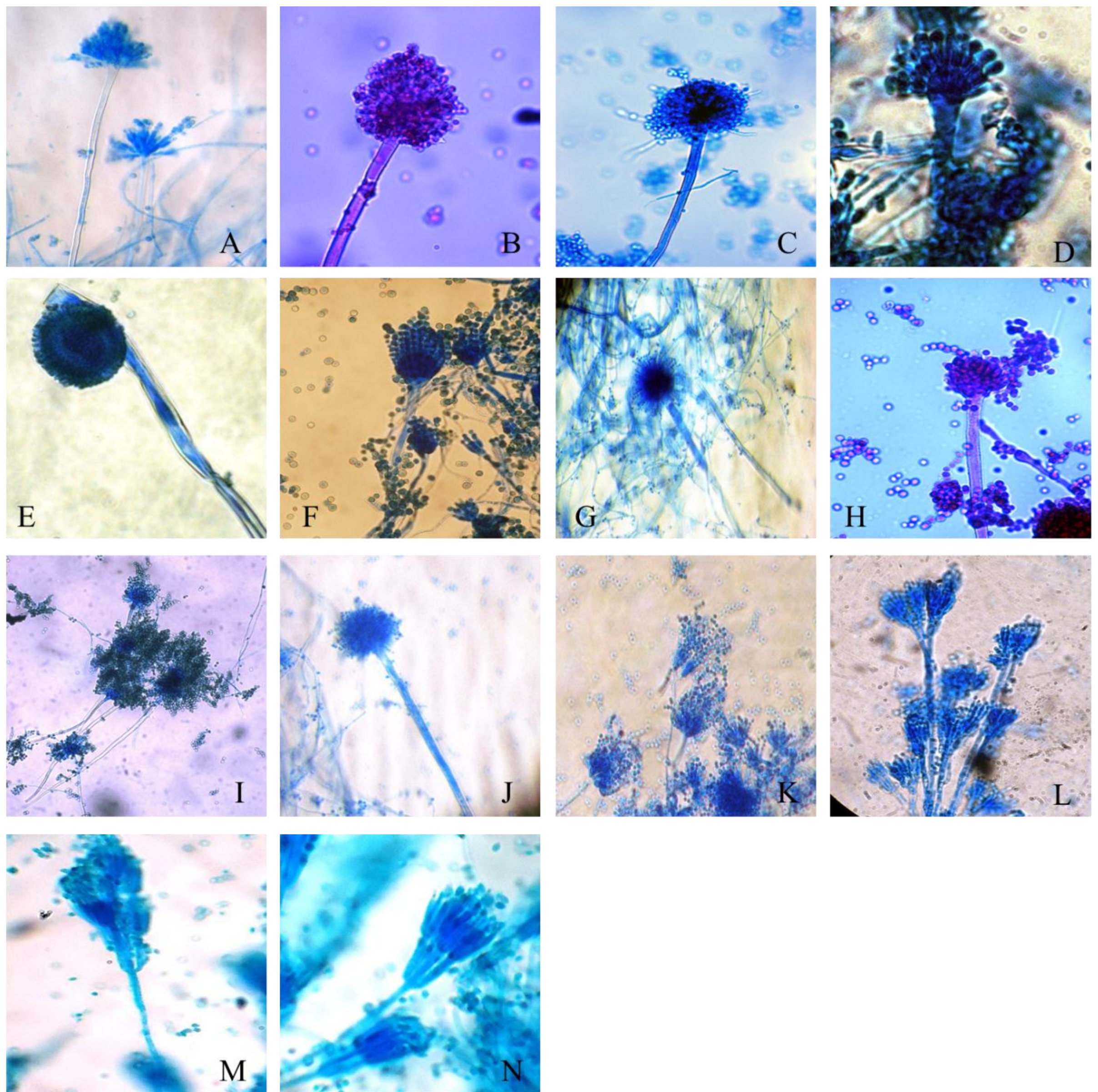


Figure 2.8 Light micrographs of fungi isolated from the guano of (A) *Aspergillus oryzae* isolate pkm8, (B) *Aspergillus stellatus* isolate VE6, (C) *Aspergillus stellatus* isolate Pkm12, (D) *Aspergillus sclerotiorum* isolate pkm9, (E) *A. niger*, (F) *Aspergillus sydowii* strain PKY1, (G) *Aspergillus caelatus* isolate pkm10, (H) *Aspergillus* sp.PKM17, (I) *Aspergillus* sp. PKM22, (J) *Aspergillus* sp. Ambedkar, (K) *Penicillium citrinum* isolate pkm1, (L) *P. citrinum* isolate pkm6, (M) *P. oxalicum* isolate VE11 and (N) *P. oxalicum* isolate pkm7.

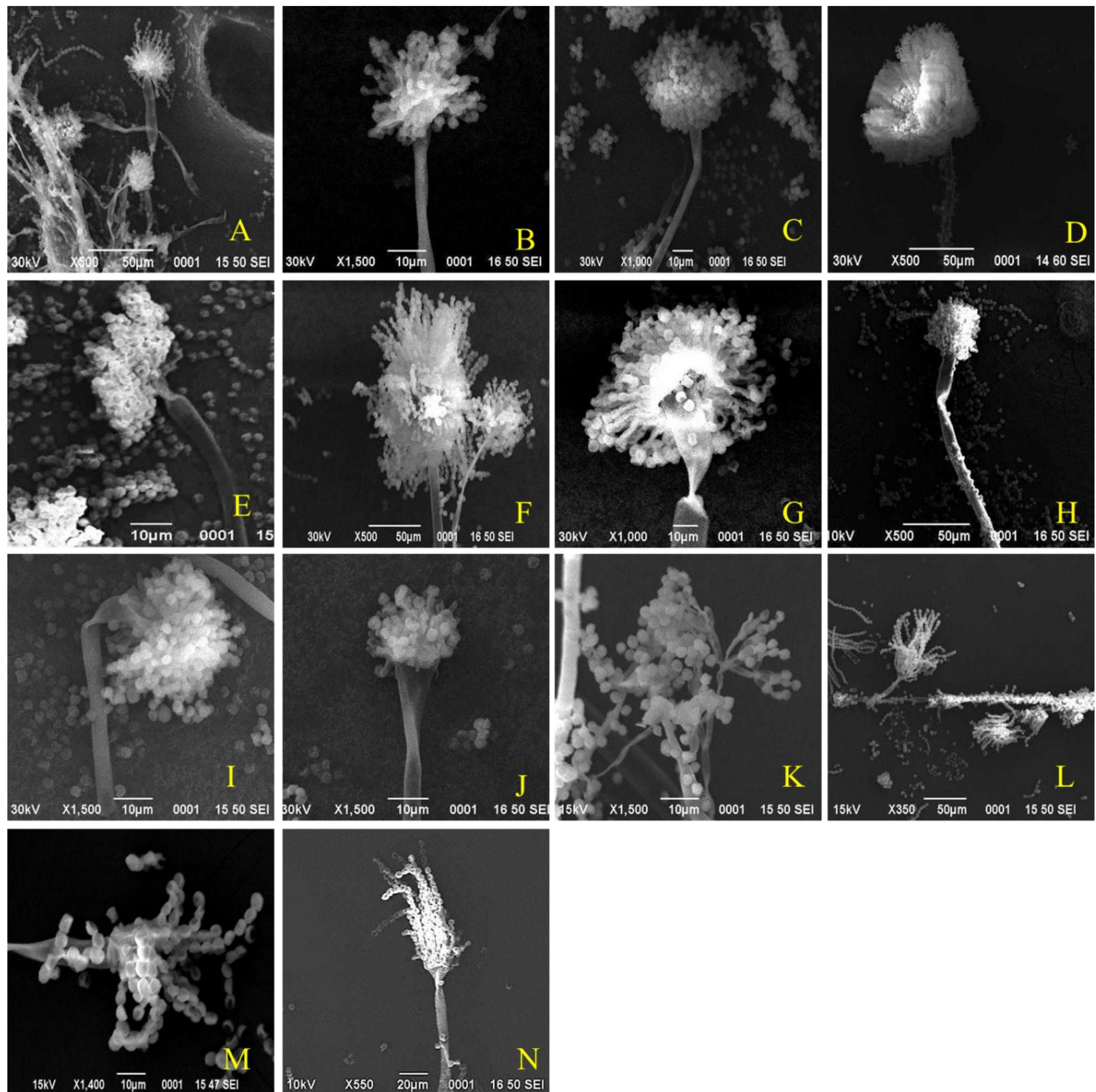


Figure 2.9 Scanning Electron micrographs of fungi isolated from the guano (A) *A. oryzae* isolate pkm8, (B) *A. stellatus* isolate VE6, (C) *A. stellatus* isolate Pkm12, (D) *A. sclerotiorum* isolate pkm9, (E) *A. niger*, (F) *A. sydowii* strain PKY1, (G) *A. caelatus* isolate pkm10, (H) *Aspergillus* sp. PKM17, (I) *Aspergillus* sp. PKM22, (J) *Aspergillus* sp. *Ambedkar*, (K) *Penicillium citrinum* isolate pkm1, (L) *P. citrinum* isolate pkm6, (M) *P. oxalicum* isolate VE11 and (N) *P. oxalicum* isolate pkm7.

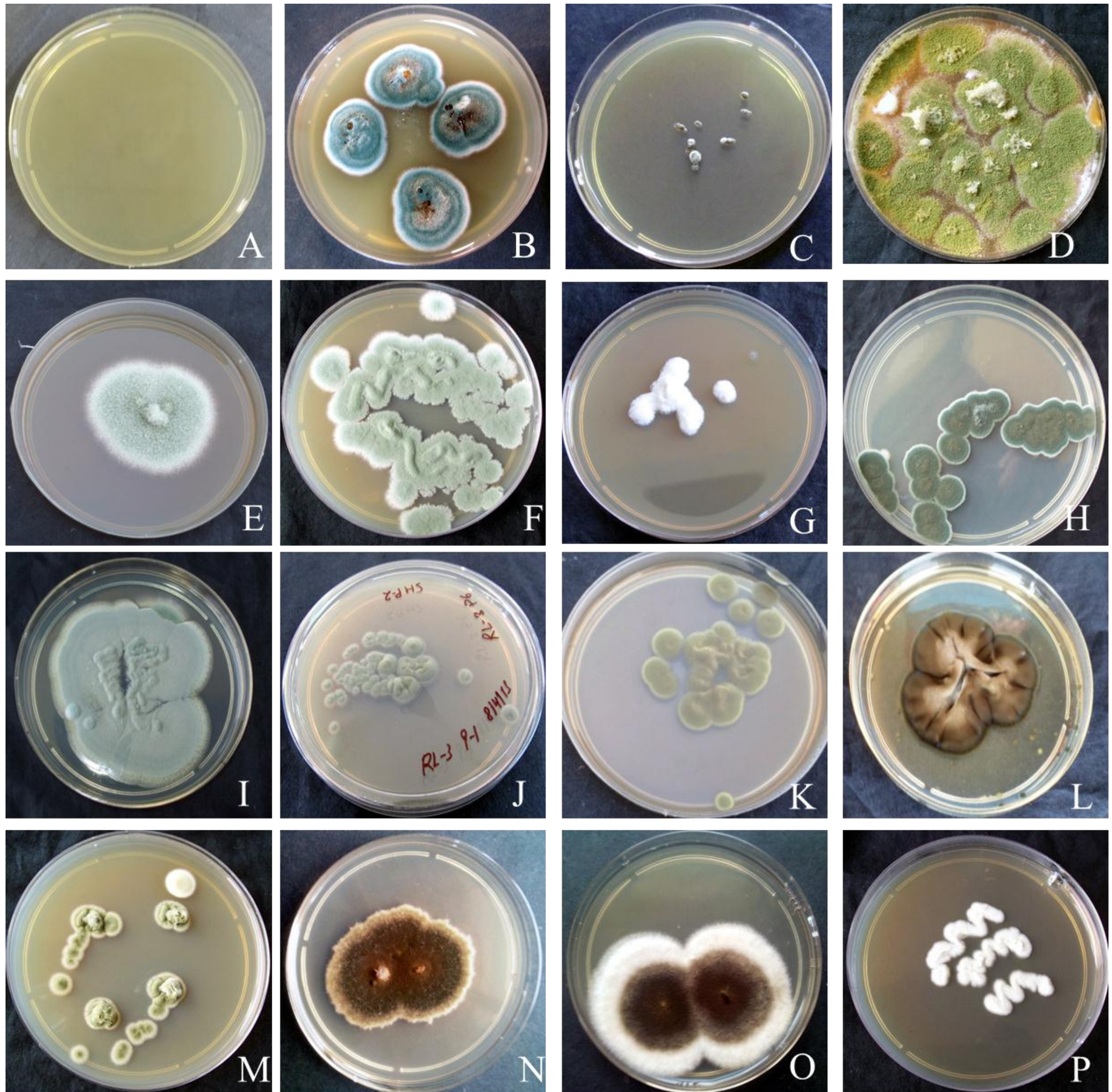


Figure 2.10 Colony morphology of fungi isolated from the guano of bats (A) Control, (B) *P. crustosum* strain PKM19, (C) *P. polonicum* isolate Pkm13, (D) *P. capsulatum* isolate pkm14, (E) *P. funiculosum*, (F) *P. concentricum* isolate VE4, (G) *P. rubidurum* isolate pkm5, (H) *Penicillium* sp., (I) *Penicillium* sp., (J) *Cladosporium tenuissimum* isolate VE8, (K) *C. cladosporiodes*, (L) *C. resinae*, (M) *Cladosporium* sp.VE3, (N) *Alternaria tenuissima* strain NKG1, (O) *Alternaria* sp. NKG2 and (P) *Mucor indicus* isolate BBAU.

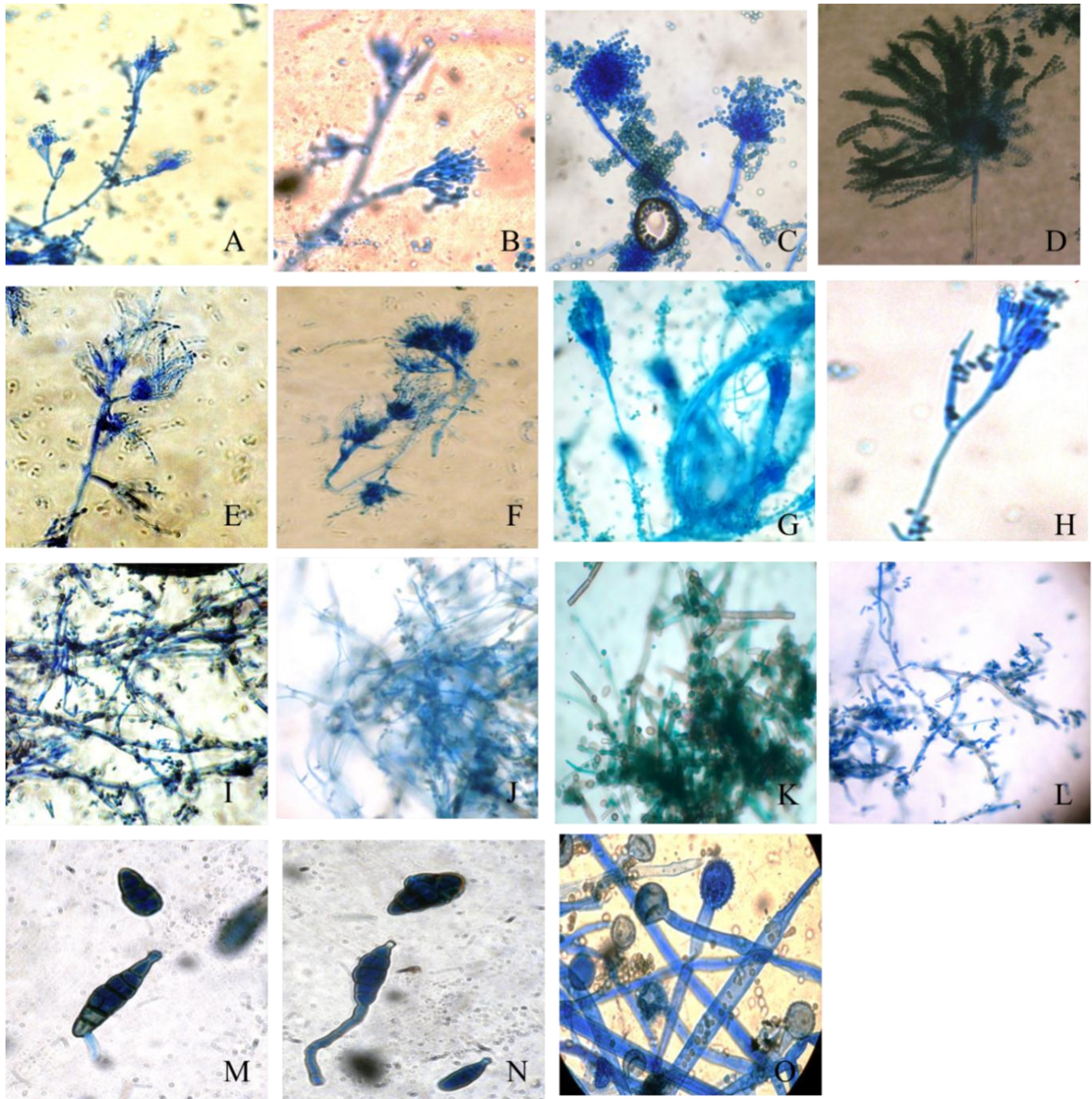


Figure 2.11 Light micrographs of fungi isolated from the guano of (A) *P. crustosum* strain PKM19, (B) *P. polonicum* isolate Pkm13, (C) *P. capsulatum* isolate pkm14, (D) *P. funiculosum*, (E) *P. concentricum* isolate VE4, (F) *P. rubidurum* isolate pkm5, (G) *Penicillium* sp., (H) *Penicillium* sp., (I) *Cladosporium tenuissimum* isolate VE8, (J) *C. cladosporiodes*, (K) *C. resinae*, (L) *Cladosporium* sp. VE3, (M) *Alternaria tenuissima* strain NKG1, (N) *Alternaria* sp. NKG2 and (O) *Mucor indicus* isolate BBAU.

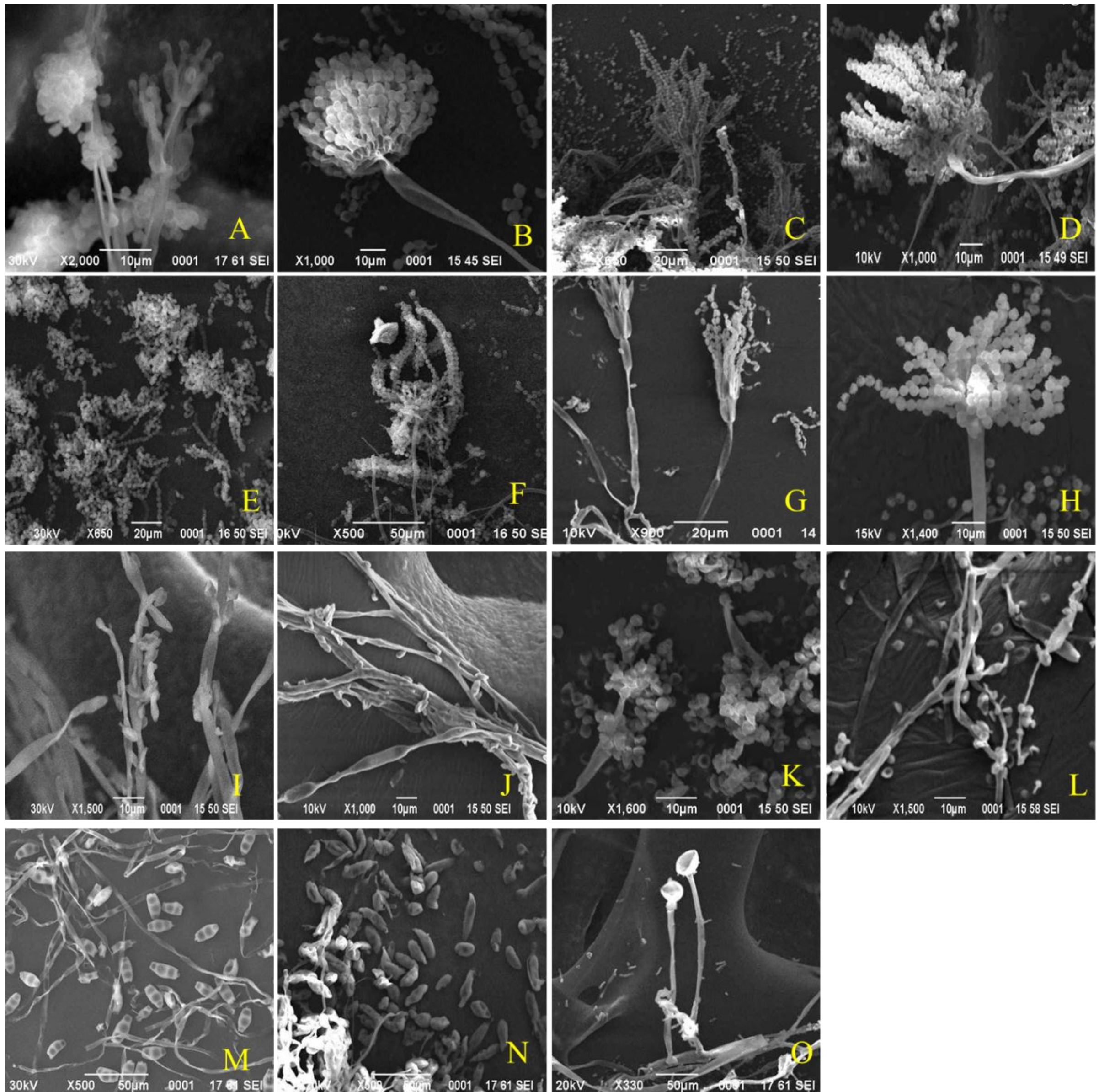


Figure 2.12 Scanning Electron micrographs of fungi isolated from the guano (A) *P. crustosum* strain PKM19, (B) *P. polonicum* isolate Pkm13, (C) *P. capsulatum* isolate pkm14, (D) *P. funiculosum*, (E) *P. concentricum* isolate VE4, (F) *P. rubidurum* isolate pkm5, (G) *Penicillium* sp., (H) *Penicillium* sp., (I) *Cladosporium tenuissimum* isolate VE8, (J) *C. cladosporiodes*, (K) *C. resinae*, (L) *Cladosporium* sp. VE3, (M) *Alternaria tenuissima* strain NKG1, (N) *Alternaria* sp. NKG2 and (O) *Mucor indicus* isolate BBAU.

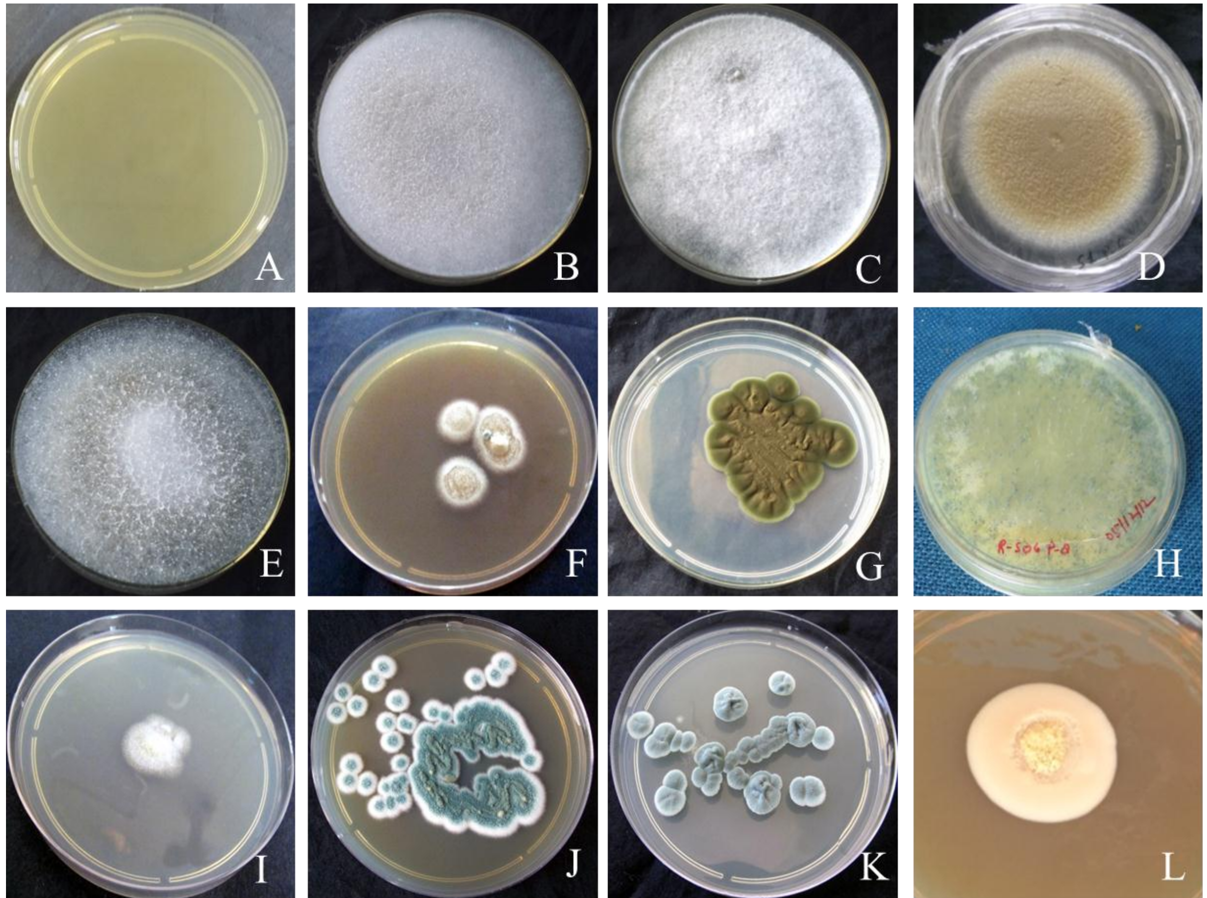


Figure 2.13 Colony morphology of fungi isolated from the guano of bats (A) Control, (B) *Mucor* sp., (C) *Chrysosporium tropicum*, (D) *Paecilomyces varitii*, (E) *Absidia corymbifera*, (F) *Sarocladium implicatum* isolate pkm4, (G) *Malbranchea* sp., (H) *Trichoderma* sp, (I) *Periconia* sp. VE2, (J) *Trichocomaceae* sp. PKM21, (K) *Davidiella* sp. PKM20 and (L) Yeast.

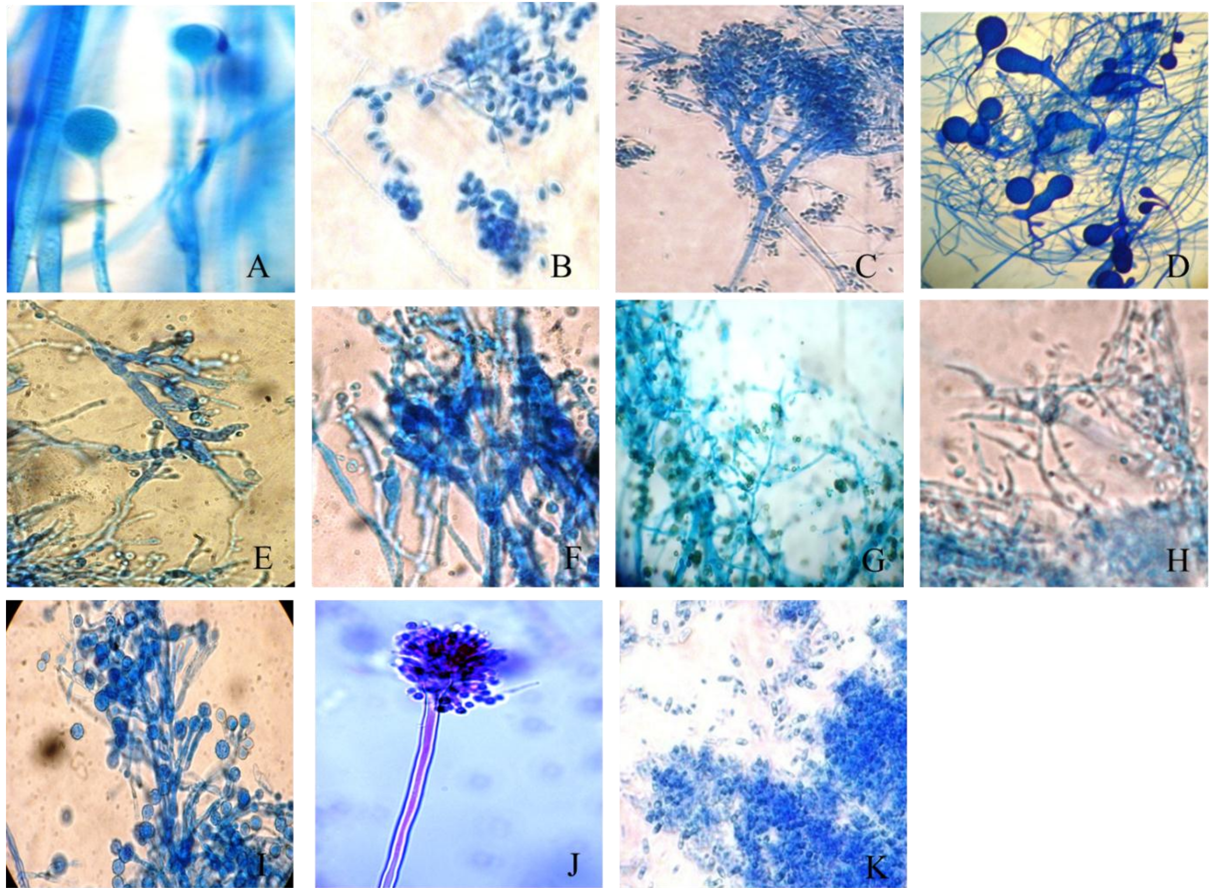


Figure 2.14 Light micrographs of fungi isolated from the guano of (A) *Mucor* sp., (B) *Chrysosporium tropicum*, (C) *Paecilomyces varitii*, (D) *Absidia corymbifera*, (E) *Sarocladium implicatum* isolate pkm4, (F) *Malbranchea* sp., (G) *Trichoderma sp*, (H) *Periconia* sp. VE2, (I) *Trichocomaceae* sp. PKM21, (J) *Davidiella* sp. PKM20 and (K) Yeast.

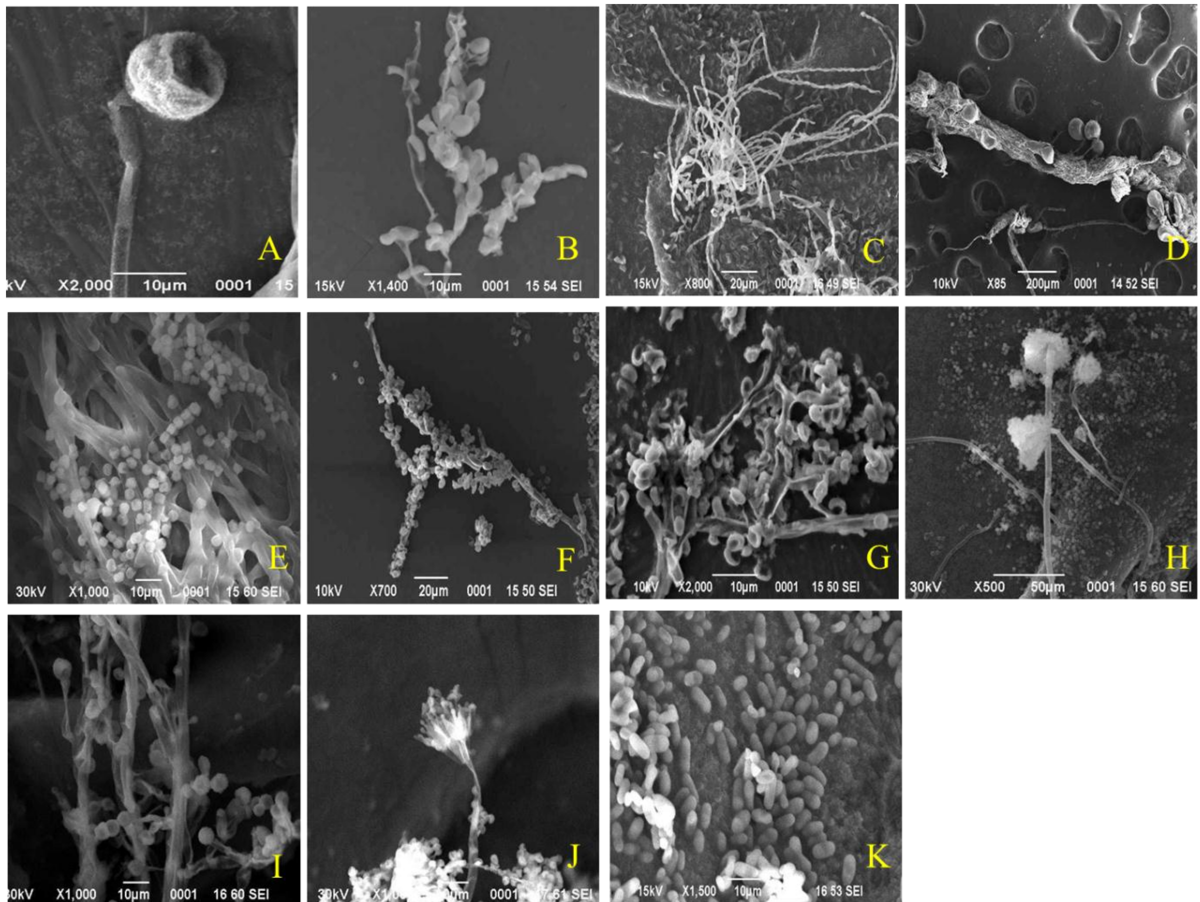


Figure 2.15 Scanning Electron micrographs of fungi isolated from the guano(A) *Mucor* sp., (B) *Chrysosporium tropicum*, (C) *Paecilomyces varitii*, (D) *Absidia corymbifera*, (E) *Sarocladium implicatum* isolate pkm4, (F) *Malbranchea* sp., (G) *Trichoderma sp*, (H) *Periconia* sp. VE2, (I) *Trichocomaceae* sp. PKM21, (J) *Davidiella* sp. PKM20 and (K) Yeast.

Chapter 03:

Characterization of guanophilic fungi using Fourier Transformed Infrared Spectroscopy (FTIR)

Chapter 03

Characterization of Guanophilic Fungi using Fourier Transformed Infrared Spectroscopy (FTIR)

Introduction

Fungi belong to diverse group of organisms comprises of five phyla, namely Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, and Glomeromycota (Kirk *et al*., 2008; Shüβler *et al*., 2001). As a practical approach to classification, fungi have been divided into groups, such as the filamentous fungi (also called moulds) Yeasts and the mushrooms.

The fungi commonly serve as saprotrophs and/or pathogens or as transient chemo heterotrophic microorganisms (Northup *et al*., 1997). Insectivorous bats are known to be the prime contenders as reservoirs of fungi such as *Histoplasma capsulatum*, *Coccidioides immitis*, *Cryptococcus laurentii*and *Blastomyces dermatitidis* (Yamamoto *et al*., 1995; Garcia Hermoso *et al*., 1997; Mattsson *et al*., 1999; Bunnell *et al*., 2000). The excreta of wild birds and animals, including bats, contain medically significant fungi, such as *Cryptococcus neoformans* and *C. laurentii* (Garcı´a-Hermoso, *et* al., 1997) and locations that contain large amounts of such excreta are potential sites of human infection.

Traditional identification of fungi is time consuming due to proper fungal culture and microscopic examination and can be influenced by the interpretation of the macro and micro morphological characters. Most commercially available identification systems used in routine are based on physiological (growth temperature) and nutritional (sugar assimilation or fermentation and enzymatic tests) characteristics. For the identification of fungi there is no physiological test is available.

Identification by using molecular technique based on sequence data is reliable source, but data are available only for certain taxa and application of molecular technique for routine analysis is relatively expensive (Fischer and Dot, 2002; Rozynek *et al.*, 2004).

By using rapid identification technique for the pathogenic fungi, time, death rates, costs associated with infectious diseases could be significantly reduced (Doern *et al.*, 1994; Maquelin *et al.*, 2003). However, available conventional methods delays in identification and limits to discriminate two closely related species.

The Fourier Transformed Infrared Spectroscopy (FTIR) has emerged as an ideal tool for the study of molecular structure and intermolecular interaction in the biological sample. Fourier transform infrared spectroscopy is based on the vibrational excitation of molecular bonds by absorption of infrared light energy. One of the strengths of IR spectroscopy is its ability as an analytical technique to obtain spectra from a very wide range of solids, liquids and gases. Traditionally IR spectrometers had been used to analyse solids, liquids and gases by means of transmitting the infrared radiation directly through the sample. The Mid-IR spectrum derives from absorption of light exciting molecular vibrations. The positions of absorption bands in the spectrum give information about the presence or absence of specific functional groups in a molecule and as a whole the spectrum constitutes a “fingerprint” that can be used to determine the identity of the sample. Many substances can be characterized, identified and also quantified. A difference between two spectra indicates that the two samples are made up of different components.

First attempts to apply IR technology for biological sample was began as early as 1910. By the late 1940, the technique was being successfully explored for the study

of biological materials and in fact, IR spectroscopy has become an accepted tool for the characterization of biomolecules (Margarita and Quinteiro, 2000).

FTIR had significantly improved in various fields such as, for the identification of bacteria, Yeasts (Schmalreck *et al.*, 1998; Timmins *et al.*, 1998), fungi, applied microbiological research, physiological properties, biodiversity studies, inventories in environmental microbiology and quality control measures (Fischer *et al*., 2005).

The use of FTIR is an analytical approach, non-destructive and dynamic method, not only to investigate a population of whole cells with only little biomass (Naumann *et al*., 1991; Maquelin *et al.*, 2003), identification and characterisation of microbial cells (Sockalingum *et al*., 1997; Sandt *et al*., 2003). This technique is increasingly being used for the identification of microorganisms at the species level, and it has been proven to have enough discriminating power to identify *C. albicans* at the strain level (Sandt *et al*., 2003). It is known fact that strains of one taxon differing in metabolite production that can be reliably distinguished by FT-IR spectroscopy. On the other hand, species from different taxa being similar in secondary metabolite production showed comparably higher similarities (Fischer *et al*., 2005). FTIR spectroscopy has been shown to be able to detect these weak variations of the cell composition which are due to changes in culture conditions (culture time, medium composition).

The aim of this study was to create a simple, routine analysis but sophisticated method for the identification of fungi using FT-IR spectroscopy.

Materials and Methods

Sample collection

Sample preparation for FT-IR spectroscopy

The specimens were cultured on plates of Sabouraud-dextrose agar with chloramphenicol and cycloheximide (actidione). The cultures plates were incubated 2 – 4 weeks at 25 °C or until mature growth became visible.

Cells were carefully harvested from sabouraud-dextrose agar plate. About 2 mg of each sample was ground into fine particles and mixed with 100 mg potassium bromide (KBr) using mortar and pestle. The mixture was dried for 2 h in a microfuge tube. The KBr based pellets were prepared by establishing pressure of 10 $kg/cm^2$for about 30 sec. The KBr tablet was used as a blank for background subtraction. The sample pellet was prepared by adding the adequate sample with the KBr and the pellet was prepared under the same pressure, to eliminate the possibility of any changes which might occur in the thickness of the pellet. A Nicolet 670 FT-IR spectrometer (Madison, WI, USA) was used for analysis. The sample compartment in the FT-IR spectrometer was continuously purged with dry air to prevent water vapour. Pellets were scanned at 4 cm^{-1} resolution with 100 scans in the spectral range of 4000 - 400 cm^{-1} at room temperature. The system was operated using the OMNIC 5.3 software and the experiments were replicated three times. Spectra belonging to the identification of the same experimental groups, baseline correction, normalization and the band areas were obtained. When they showed identical curves, they were considered as the reference curve and compared the rest of cases of the same species according to culture results with that reference curve. Finally, the reference curves of all isolated species in this study had been compared together. The obtained peak frequency was assessed by

following Dyer (1965).The fingerprint region of Fourier Transformed Infrared Spectroscopy (FTIR) is 4000 – 400 cm^{-1} but the peak below 1100 cm^{-1} not taken in consideration because the peak are marched to each other and don't follow hook law, so that I took the peak above 1100 cm^{-1}.

Results

The FTIR spectra of fungal isolates were stacked and given in Figures 3.1 – 3.11. The wavenumbers of FTIR spectra, type of oscillation, fungal isolates and details of functional groups are given in Table 3.1. The IR absorption spectra found from wave number 3440.1 cm^{-1} to 3377.2 cm^{-1} in *A. flavus* strain PKM24, *A. flavus* isolates pkm11 and VE7, *A. versicolar gr.*, *A. versicolor* strains PKM16 and PKM25, *A. oryzae* isolates pkm8 and VE9, *A. stellatus* isolate Pkm12, *A. sclerotiorum* isolate pkm9, *Aspergillus* sp. PKM22, *P. citrinum* isolate pkm6, *P. oxalicum* isolate pkm7, *P. capsulatum* isolate pkm14, *P. concentricum* isolate VE4, *P. rubidurum* isolate pkm5, *C. resinae*, *P. varitii*, *Malbranchea* sp., *Cladosporium* sp. VE3, *Mucor indicus* isolate BBAU, *Malbranchea* sp. and *Trichoderma* sp. due to the stretching of bonded O–H and the presence of phenol and alcohols.

Out of 41 fungal isolates, N–H stretching between 3395.2 cm^{-1} and 3297.8 cm^{-1} was observed in 19 fungal isolates such as *A. flavus* strain PKM18, *A. versicolor* strain PKM25, *A. versicolar gr.*, *A. oryzae* isolate pkm8, *A. oryzae* isolate VE9, *A. sydowii* strain PKY1, *A. stellatus* isolate VE6, *A. niger*, *Aspergillus* sp. PKM17, *P. funiculosum*, *P. oxalicum* isolate VE11, *Penicellium* sp., *C. cladosporiodes*, *C. tenuissimum* isolate VE8, *C. tropicum*, *A. corymbifera*, *Davidiella* sp. PKM20, *Trichocomaceae* sp. PKM21, *Periconia* sp. VE2, *Mucor* sp. and Yeast which showed the presence of imines (Table 3.1).

The wave number 3292.3 – 3272 cm^{-1} was with weak and broad peak only observed in *A. flavus*, *A. flavus* isolate pkm2due to O–H stretching and presence of chelate compound of alcohols and phenols.

The absorption band from 2936.2 cm^{-1} to 2880.1 cm^{-1} observed in all fungal isolates except *P. capsulatum* isolate pkm14 due to C–H stretching showed the presence of alkene (aromatic).

The IR band from 2364.9 cm^{-1} to 2345.7 cm^{-1} was observed in 24 fungal isolates such as *A. flavus*, *A. flavus* strains PKM18 and PKM24, *A. flavus* isolates pkm11 and VE7, *A. versicolar gr.*, *A. versicolor* strains PKM16 and PKM25, *A. sydowii* strain PKY1, *A. stellatus* isolates Pkm12 and VE6, *A. sclerotiorum* isolate pkm9, *Aspergillus* sp. PKM17, *P. citrinum* isolate pkm6, *P. oxalicum* isolate pkm7, *P. rubidurum* isolate pkm5, *Penicillium* sp., *C. cladosporiodes*, *C. resinae, C. tropicum*, *Davidiella* sp. PKM20, *Trichocomaceae* sp. PKM21, *M. indicus* isolate BBAU, *Malbranchea* sp. and *Trichoderma* sp.due to stretching of C–N showed the presence of unsaturated nitrogen compound.

Due to C–C triple bond IR bands observed from 2247.8 cm^{-1} – 2083.8 cm^{-1} in *Aspergillus* sp. PKM17 and *C. tenuissimum* isolate VE8 showed the presence of mono-substituted alkyne.

The IR band from 1747.1 cm^{-1} to 1631.3 cm^{-1} due to stretching of C–C multiple bonds observed in all fungal isolates showed the presence of alkyne.

The absorption bands from 1552.2 cm^{-1} – 1522.1 cm^{-1} observed in many fungal isolates except *A. flavus* strain PKM24, *P. citrinum* isolate pkm6, *P. capsulatum* isolate pkm14, *P. rubidurum* isolate pkm5, *A. corymbifera*, *Cladosporium* sp. VE3, *M.*

indicus isolate BBAU and *Malbranchea* sp. showed the presence of secondary amide (dilute solution).

Due to C–H bending from 1465 cm^{-1} – 1428.6 cm^{-1} in *A. flavus*, *A. flavus* isolate pkm11, *A. versicolar gr.*, *P. citrinum* isolate pkm6, *P. oxalicum* isolate VE11, *P. rubidurum* isolate pkm5, *P. varitii*, *Cladosporium* sp. VE3, *C. cladosporiodes*, *Mucor* sp., *Malbranchea* sp. and Yeast fungal isolates showed the presence of alkane.

Weak C–O/ O–H bending was observed due to 1415.7 - 1400.1 cm^{-1} in *A. flavus* strains PKM18, PKM24, *A. versicolor* strain PKM25, *A. oryzae* isolate pkm8, VE9, *A. sydowii* strain PKY1, *A. niger*, *Aspergillus* sp.PKM17, *P. concentricum* isolate VE4, *Penicillium* sp., *C. resinae*, *C. tenuissimum* isolate VE8, *C. tropicum*, *P. varitii*, *A. corymbifera*, *Davidiella* sp.PKM20, *Trichocomaceae* sp. PKM21, *Periconia* sp.VE2, *Trichoderma* sp. and Yeast showed the presence of alcohols and phenols.

The IR absorption between 1382.8 and 1313.3 cm^{-1} due to strong bonding of C-NO_2 was found in *A. flavus*, *A. flavus* isolate pkm2 and VE7, *A. versicolar gr.*, *A. stellatus* isolate VE6, *A. sclerotiorum* isolate pkm9, *Aspergillus* sp. PKM22 and PKM17, *P. funiculosum*, *P. oxalicum* isolate pkm7, *Periconia* sp. VE2, *M. indicus* isolate BBAU and *Mucor* sp. showed the presence of aliphatic groups nitro compounds.

The absorption bands of 1315.3 cm^{-1} was observed only in *A. oryzae* isolate pkm8 showed methyl C-H stretching and presence of Amide II.

The strong C–O bond observed from 1256.9 cm^{-1} – 1234.2 cm^{-1} in *A. flavus* isolates pkm2, PKM18, PKM24 and VE7, strains of *A. versicolor* such as PKM16 and PKM25, *A. oryzae* isolate pkm8, *A. sclerotiorum* isolate pkm9, *A. niger*, *Aspergillus* sp. PKM17, *P. funiculosum*, *P. oxalicum* isolates pkm7 and VE11, *C. resinae*, *C.*

tenuissimum isolate VE8, *C. tropicum*, *P. varitii*, *Davidiella* sp. PKM20, *Mucor* sp. and *Trichoderma* sp. showed the presence of ether group.

The absorption bands from 1158.1 cm^{-1} to 1148.5 cm^{-1} observed due to C–O–C symmetrical stretching in only few fungal isolates such as *A. flavus*, *A. versicolor* strain PKM25, *A. stellatus* isolate Pkm12, *A. sclerotiorum* isolate pkm9, *P. oxalicum* isolate pkm7, *C. resinae*, *C. tenuissimum* isolate VE8, *C. tropicum*, *P. varitii*, *A. corymbifera*, *Cladosporium* sp. VE3, *Periconia* sp. VE2 and *Mucor* sp. showed the presence of polysaccharides.

Discussion

In the current study Fourier-transform infrared (FTIR) spectroscopy was used and observed that FTIR spectroscopy is a sensitive method for the investigation of fungi biochemical composition. This is a rapid and reproducible method for fungal characterization. All fungal isolates/strains were cultivated using the same culture medium and at the same temperature. Therefore the changes in wavenumber due to oscillation of chemical functional group of fungi.

Cell wall of fungi are mainly comprised of glycoproteins and polysaccharides (Glucan and chitin), minor cell wall components are present and vary amongst species of fungi. Presence of several –OH group represent the presence phenol and alcohols that are characteristic feature of chitin. In the cell wall of filamentous fungi such as *A. flavus*, *A. versicolor*, *A. oryzae*, *A. stellatus*, *A. sclerotiorum* isolate pkm9, *Aspergillus* sp., *P. citrinum*, *P. oxalicum*, *P. capsulatum*, *P. concentricum*, *P. rubidurum*, *C. resinae*, *P. varitii*, *Malbranchea* sp., *Cladosporium* sp., *M. indicus*, *Malbranchea* sp.and *Trichoderma* sp. several –OH were found because cell wall of filamentous fungi is composed of chitin. Chitin in the cell walls of filamentous fungi, such as *Neurospora*

and *Aspergillus* were reported to contain 10–20% chitin (Nobel *et al.*, 2000; Bowman and Free, 2006).

Chitin is a long linear homopolymer of beta-1, 4-linked N-acetylglucosamine, is considered to be a relatively minor, yet structurally important, component of the fungal cell wall (Santos *et al.*, 2000). Imines were observed in 19 fungal isolates which belong to genus *Aspergillus*, *Penicellium*, *Cladosporium*, *Chrysosporium*, *Absidia*, *Davidiella*, *Trichocomaceae*, *Periconia* and *Mucor* due to presence of alpha-chitin (Dyer, 1965),

It is interesting to tell that basically C–N group is presenting at peak 2250 cm^{-1}, we received peak at ≈2300 cm^{-1}due to attachment of C=O with C-N group because cell wall of fungus is made of chitin, which is composed of C=O and C–N group. In few fungal strains this peak is not available and assumed that C–N group is not present in those strains of fungal cell wall.

Acetyl ester bonds (1747.1 cm^{-1} to 1631.3 cm^{-1}) were observed in all fungal isolates due to stretching of C–C multiple bonds.

The presence of N-acetyl ester bonds due to amide II is the characteristic feature of the cell wall of fungi (Dyer, 1965). The ester bonds were observed in all fungal isolates/strains except the following eight isolates such as *A. flavus* strainPKM24, *P. citrinum* isolate pkm6, *P. capsulatum* isolate pkm14, *P. rubidurum* isolate pkm5, *A. corymbifera*, *Cladosporium* sp. VE3, *M. indicus* isolate BBAU and *Malbranchea* sp.

All fungal isolates showed the spectral peak between 1747.1 cm^{-1} and 1631.3 cm^{-1}, as well as near 3265, 3105, 1655, 1620 and 1550 cm^{-1} are characteristics of chitin; the band at 1590 cm^{-1} appears to be the most distinctive one for chitosan (Michell and Scurfield, 1970).

Cell-wall polysaccharides been used for classification of filamentous fungi (Bartinicki-Garcia, 1968), identification of Yeasts (Gorin and Spencer, 1970) and as possible aids in the classification and identification of lichen (Carbonero *et al.*, 2001). Polysaccharides are the main constituents of fungal cell walls and some of them, such as β1,3-D-glucans that also contain some β-1,6-linked branches and chitin, a linear polymer of N-acetyl-glucosamine, are distributed widely in many species of different genera (Santos *et al.*, 2000). Glycosidic linkage was observed in *A. flavus*, *A. versicolor* strain PKM25, *A. stellatus* isolate Pkm12, *A. sclerotiorum* isolatepkm9, *P. oxalicum* isolate pkm7, *C. resinae*, *C. tenuissimum* isolate VE8, *C. tropicum*, *P. varitii*, *A. corymbifera*, *Cladosporium* sp.VE3, *Periconia* sp.VE2 and *Mucor* sp. showed the presence of saccharides structure (Lu *et al.*, 2006). The cell wall of *A. niger* consists chiefly of neutral carbohydrate (73– 83%) and hexosamine (9– 13%), with smaller amounts of lipid (2 – 7%), protein (0.5 – 2.5%) and phosphorus (less than 0.1%) (Johnston, 1965).

The obtained results here can serve as a basis for the development of a database for species identification on the basis of chemical composition and strain characterization of microfungi.

Table 3.1.Shows the wavenumbers, oscillations of functional groups (*) and guanophilic fungal isolates.

Sample code/ Functional group	O-H Stretching, (v, sh) alcohols and phenols	N-H stretching, (m) Imines	O-H Stretching, (v) vibration chelate compound, alcohols and phenols	C-H stretching, (m-s) alkene (aromatic)	C-N (bonds stretching, m) unsaturated nitrogen compound	C-C triple bond stretching, (s) monosubtituted alkyne	C-C multiple bond stretching, (v) alkene (straight chain)	N-H Bending vibration, (s) secondary amide dilute solution	C-H Bending, () alkane	C-O/ O-H (w) bending alcohols and phenols	C-NO_2, Nitro compounds, (s) aliphatic	C-H (v) stretching Amide II	C-O (s) bonded ether	C-O-C (s) symm. Stretching, Polysachhrides
Aspergillus flavus isolate *pkm2*			3292.3	2928.1			1650.1	1547.8			1378.9		1245.8	
A. flavus strainPKM18		3385		2927.6	2360.4		1660.7	1547.6		1407.6			1242.3	
A. flavus isolate *pkm11*	3435.5			2926.8	2361.4		1643.9	1548.5	1460.7					
A. flavus strainPKM24	3383.8			2936.2	2358.5		1633			1406.2			1250.8	
A. flavus isolateVE7	3385.5			2931.3	2361.8		1651.9	1548.4			1378.7		1235.9	
A. flavus			3272	2880.1	2364.9		1661.8		1465		1372.9			1156.5
A. versicolor strainPKM16	3419.2			2930.5	2357.4		1650.2	1552.2					1244.5	
A. versicolor strainPKM25		3378.6		2929.1			1655.1	1550.9		1404.9			1239	1150.7

A. versicolar gr.		3394	2925.2	2361.7		1651.4	1548.7	1456.9		1376.7			
A. oryzae isolate pkm8		3375.9	2926.1			1650.5	1546.5		1401.8		1315.5	1239.1	
A. oryzae isolate VE9		3370	2925.5			1651.3			1409				
A. sydowii strain PKY1		3381.4	2928.9	2361.6		1652.8	1547.5		1407.8			1240.9	
A. stellatus isolate Pkm12	3377.2		2929.3	2360.2		1651	1548.98			1371.1			1153.4
A. stellatus isolate VE6		3386	2929.6	2360.1		1651.7	1548.4			1378.1			
A. sclerotiorum isolate pkm9	3382.7		2930.9	2363.1		1651.4	1548.6			1381.1		1242.5	1150.3
A. niger		3379.5	2928			1650.6	1550.3		1410.2			1252.9	
Aspergillus sp. PKM22	3401.7		2927.2			1650.4	1548.3			1378.1			
Aspergillus sp. PKM17		3386.3	2930.9	2362.8	2083.8	1660.6	1546.4		1400.5	1351.9		1238.6	
Penicillium funiculosum		3393.1	2927.5			1655.6	1550.8			1379.3		1245.3	
P. citrinum isolate pkm6	3438.4		2931	2360.1		1639.7		1456.2					
P. oxalicum isolate pkm7	3398.4		2932	2361.4		1651.9	1548.8			1376.9		1239.9	1152.2

P. capsulatum isolate pkm14	3440.1				1641.7					
P. concentricum isolate VE4	3403.2		2933.5		1648.6	1544.6		1415.7		
P. oxalicum isolate VE11		3324.7	2927.6		1648.9	1545	1456.2		1245.7	
P. rubidurum isolate pkm5	3431.8		2925.5	2362	1646.5		1459.9			
Penicillium sp.		3386	2928	2361.8	1649.9	1543.8		1404.1		
Cladosporium cladosporiodes		3392.7	2925.8	2362.5	1648.1	1545.3	1459.3			
C. resinae	3404.8		2924.7	2361.6	1642.3			1412	1239.9	1153.4
C. tenuissimum isolate VE8		3371.5	2884.5		1651.2	1544.9		1400.1	1242.7	1153.5
Chrysosporium tropicum		3297.8	2928.2	2361.8	1654.7	1548		1414.7	1238.5	1149.1
Paecilomyces varitii	3414.1		2925.2		1647.2	1551.3	1457.2	1404	1247.8	1149.7
Absidia corymbifera		3391.9	2925.8		1643			1408.3		1152.1

Cladosporium sp. VE3	3418.7		2924.5		2247.8	1633.5		1459					1157.2
Davidiella sp. PKM20		3377.3	2929	2362.2		1652.8	1544.7		1404			1240.9	
Trichocomaceae sp. PKM21		3385.6	2925.1	2361.7		1650.7	1549.6		1411.5				
Periconia sp. VE2		3378.1	2930.8			1657.3	1551.2		1406.2	1313.3			1153.6
Mucor indicus isolate BBAU	3420.7		2926.4	2362.2		1636.9				1382.8			
Mucor sp.		3390.1	2935.3			1654.9	1549.5	1428.6		1375.8		1256.9	1154.9
Malbranchea sp.	3425.1		2924.6	2345.7		1631.3		1456.6					
Trichoderma sp.	3422		2925.2	2360.1		1643.3	1548.2		1411.7			1234.2	
Yeast		3395.2	2928.6			1747.1	1522.1	1461.9		1346.6			1158

*(v = variable, s = strong, m = medium, sh = sharp w = weak and s = strong)

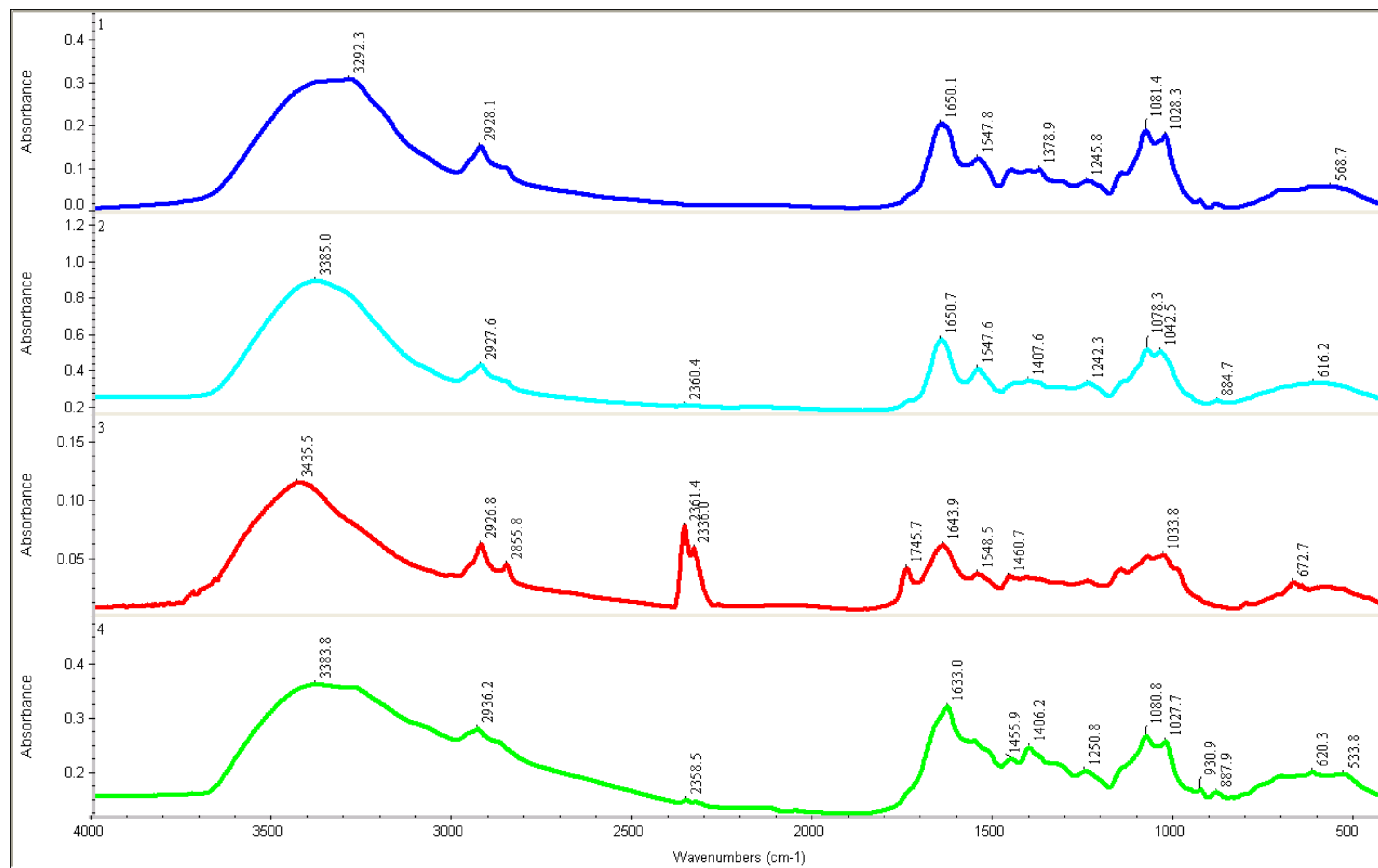


Figure 3.1 FTIR absorbance spectra of guanophilic fungi such as *A. flavus* isolate pkm2 (1), *A. flavus* strain PKM18 (2), *A. flavus* isolate pkm11 (3) and *A. flavus* strain PKM24 (4).

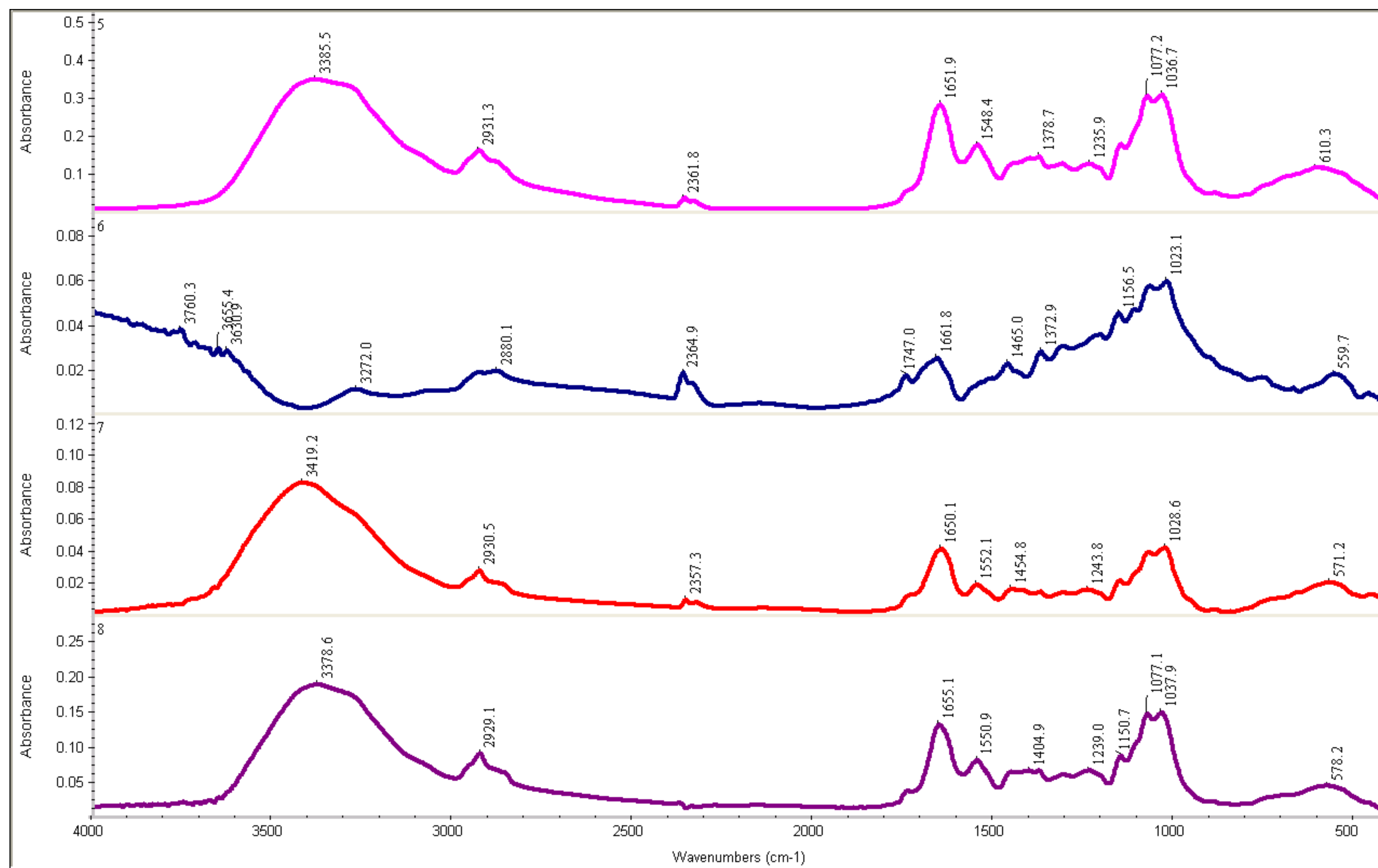


Figure 3.2 FTIR absorbance spectra of guanophilic fungi such as *A. flavus* isolateVE7 (5), *A. flavus* (6), *A. versicolor* strain PKM16 (7) and *A. versicolor* strain PKM25 (8).

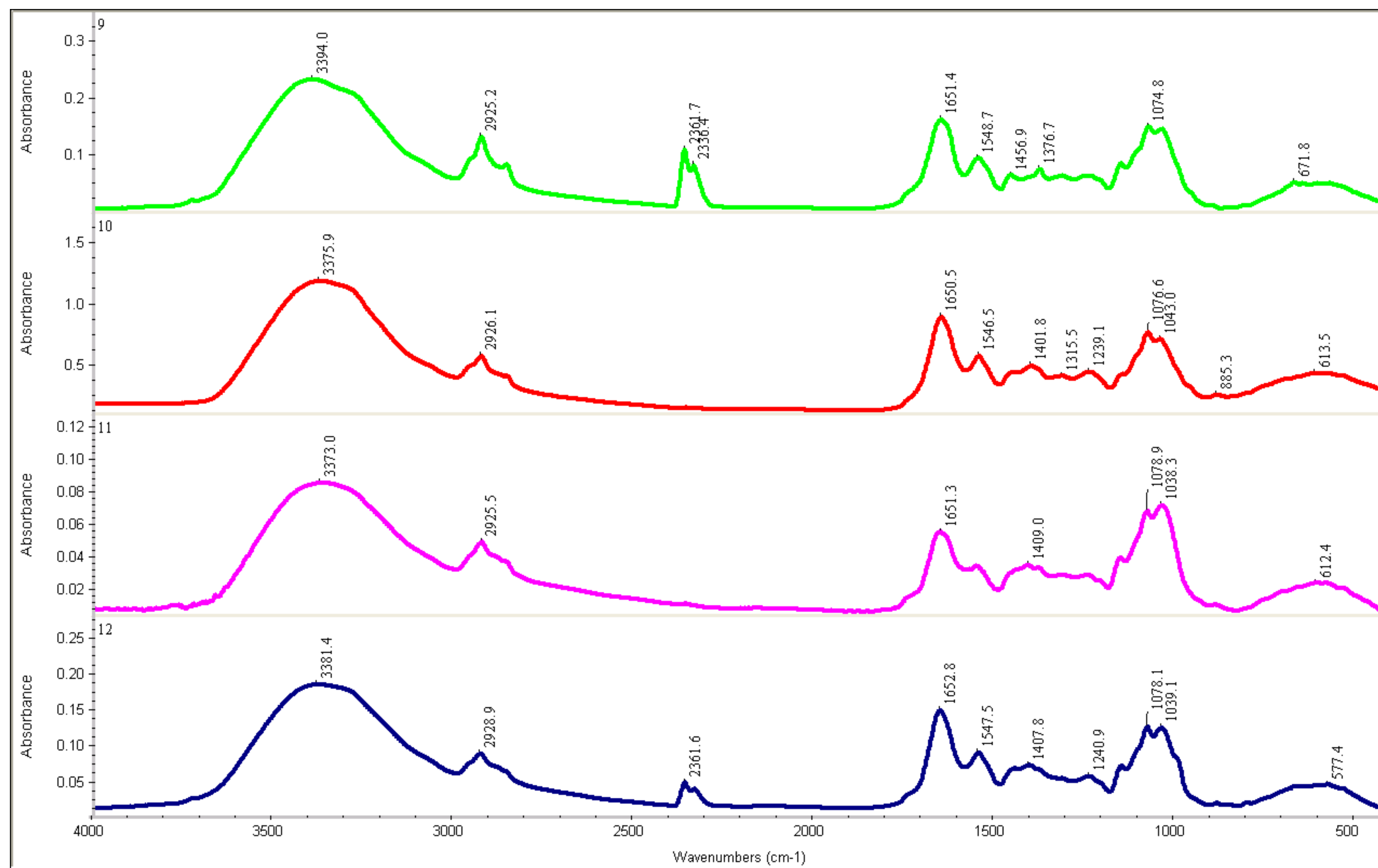


Figure 3.3 FTIR absorbance spectra of guanophilic fungi such as *A. versicolar* gr. (9), *A. oryzae* isolate pkm8 (10), *A. oryzae* isolate VE9 (11) and *A. sydowii* strain PKY1 (12).

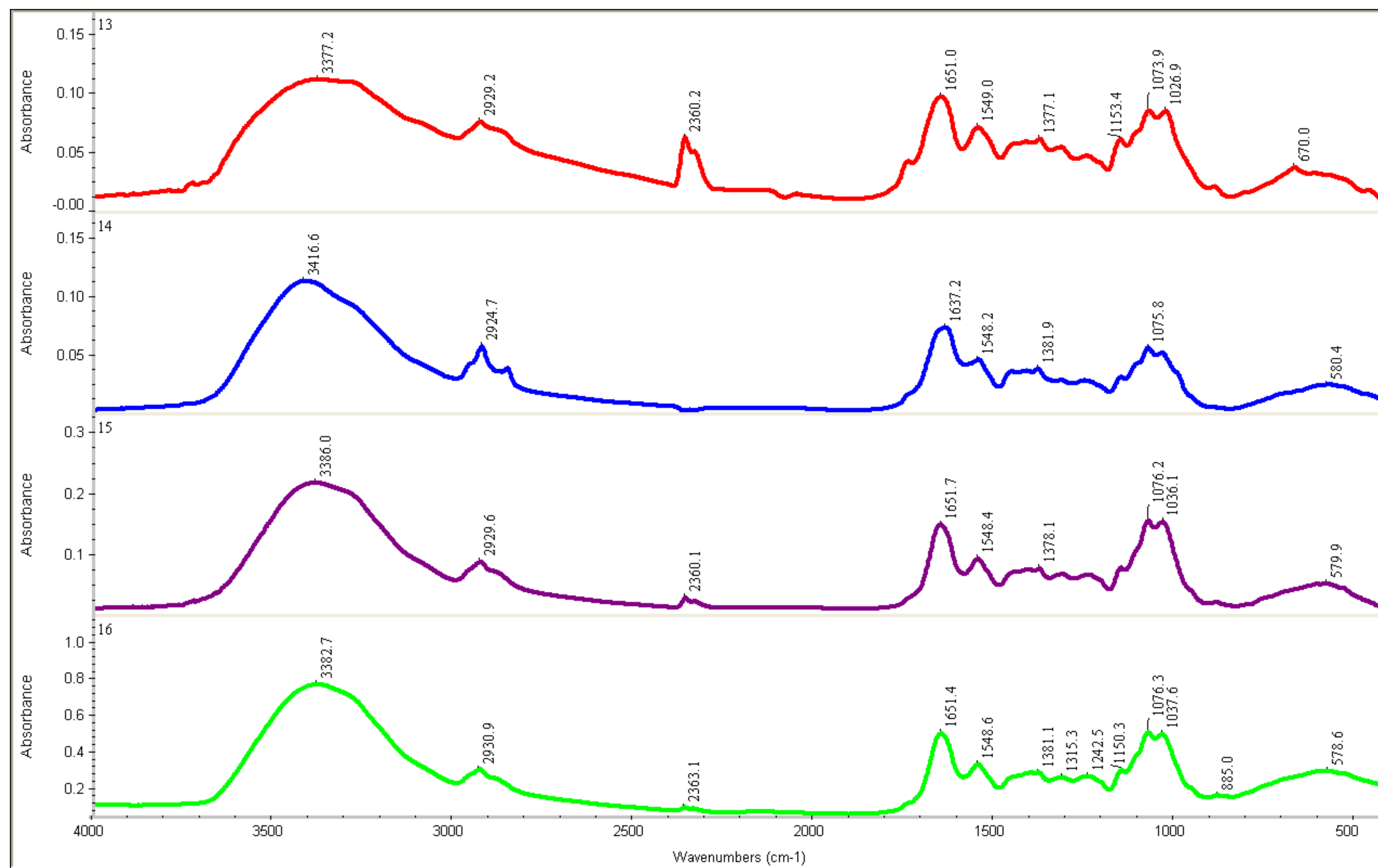


Figure 3.4 FTIR absorbance spectra of guanophilic fungi such as *A. stellatus* isolate Pkm12 (13), *A. stellatus* isolate VE6 (14), *A. stellatus* isolate VE6 (15) and *A. sclerotiorum* isolate pkm9 (16).

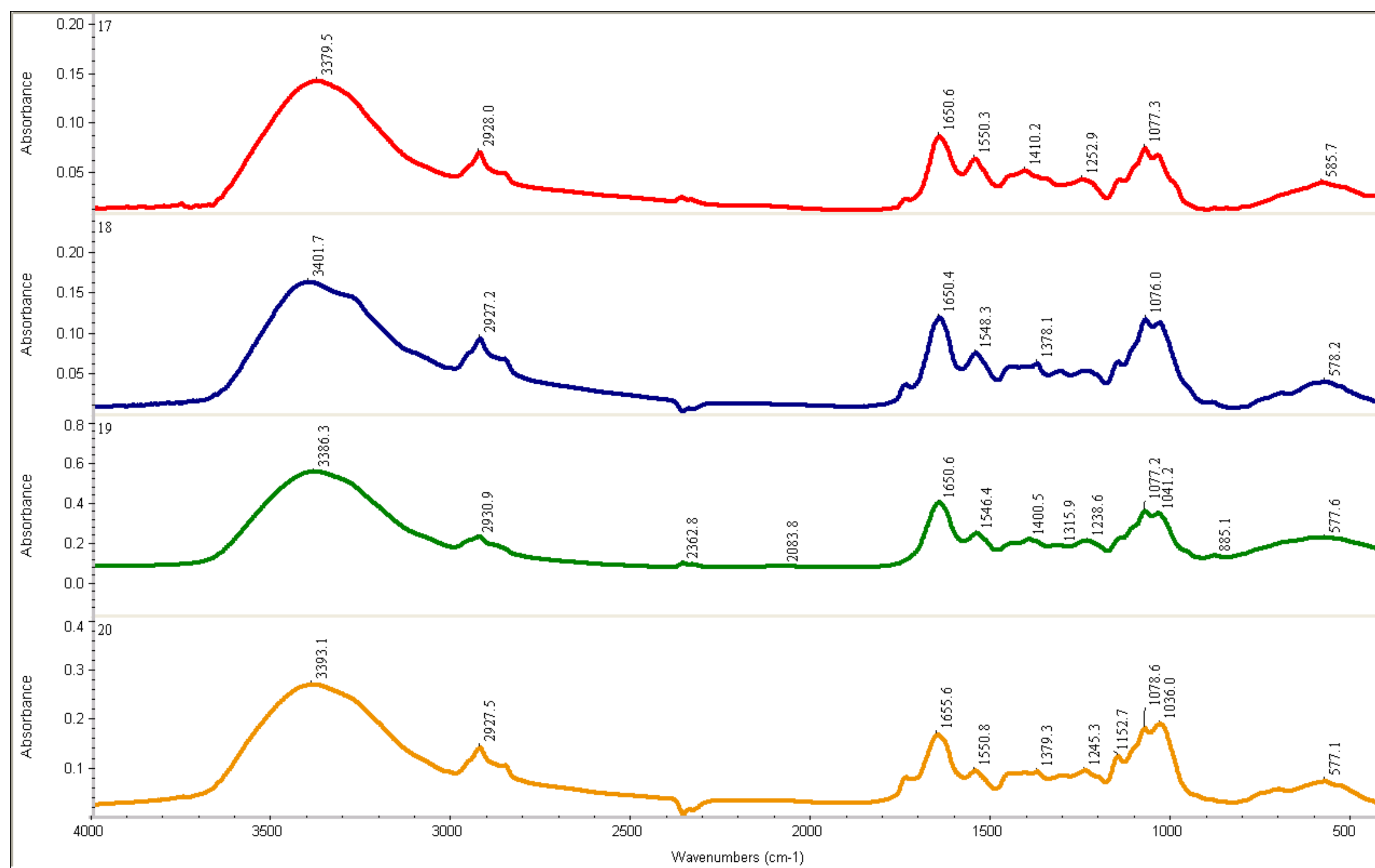


Figure 3.5 FTIR absorbance spectra of guanophilic fungi such as *A. niger* (17), *Aspergillus* sp. PKM22 (18), *Aspergillus* sp.PKM17 (19) and *P. funiculosum* (20).

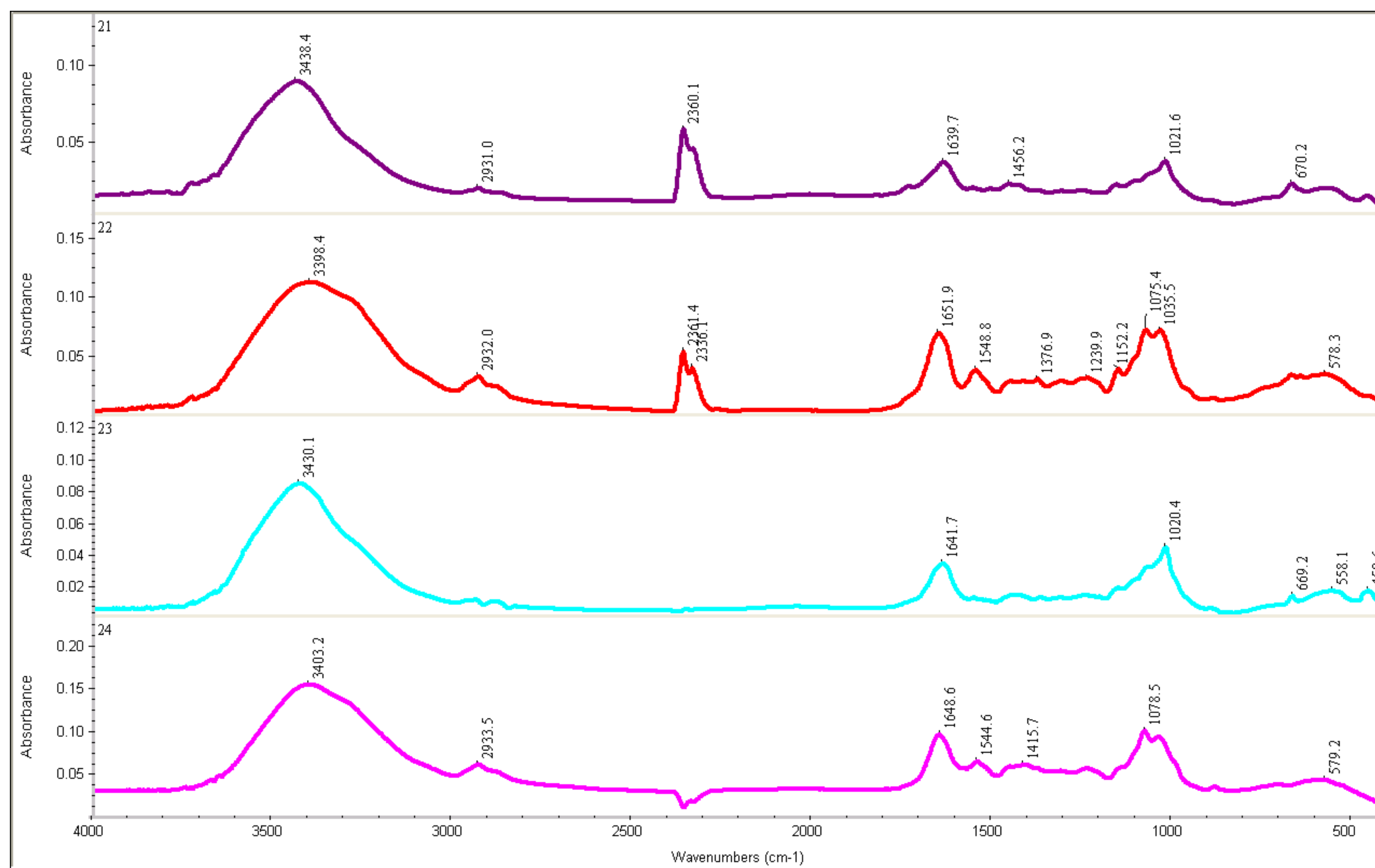


Figure 3.6 FTIR absorbance spectra of guanophilic fungi such as *P. citrinum* isolate pkm6 (21), *P. oxalicum* isolate pkm7 (22), *P. capsulatum* isolate pkm14 (23) and *P. concentricum* isolate VE4 (24).

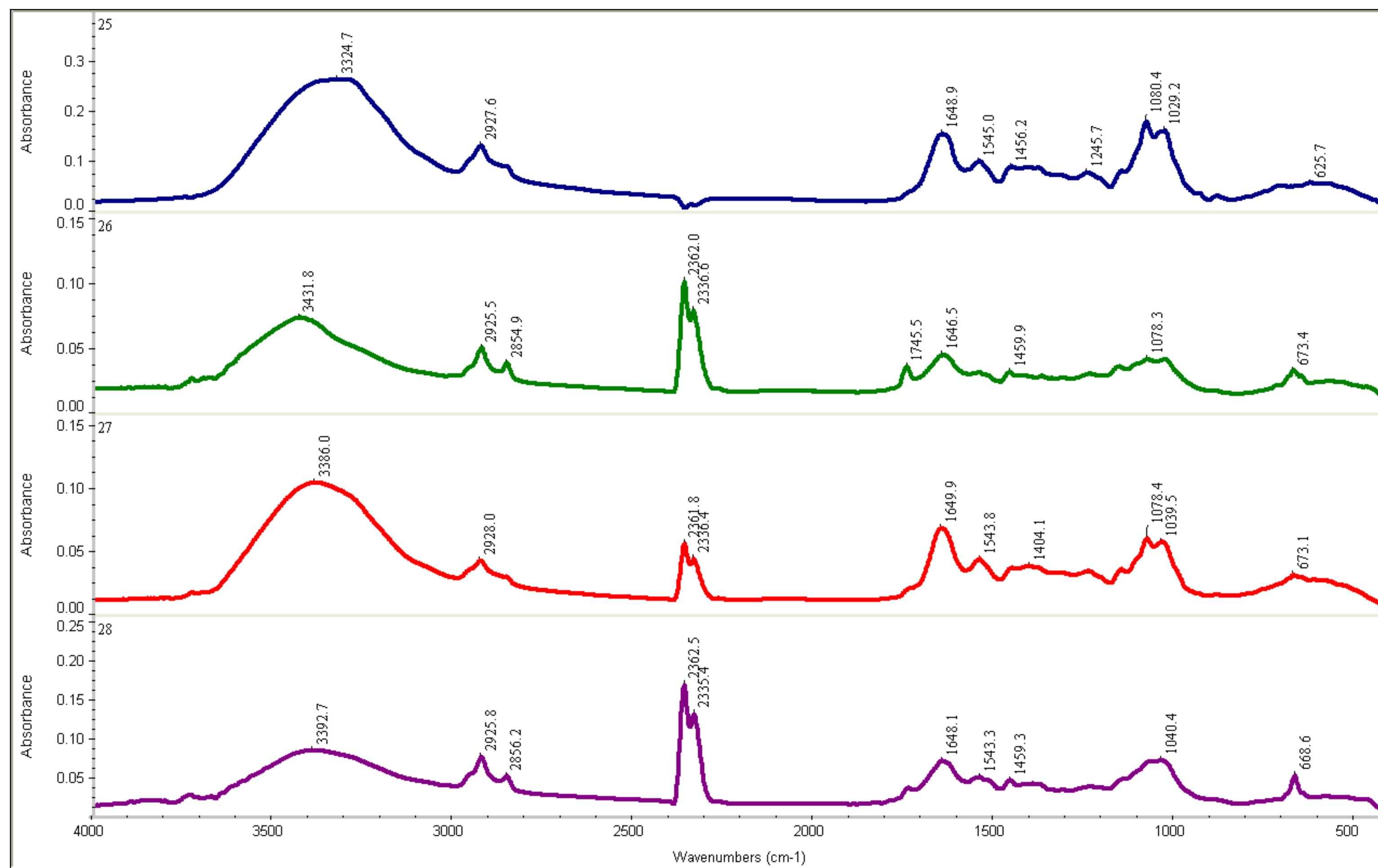


Figure 3.7 FTIR absorbance spectra of guanophilic fungi such as *P. oxalicum* isolate VE11 (25), *P. rubidurum* isolate pkm5. (26), *Penicellium* sp. (27) and *C. cladosporiodes* (28).

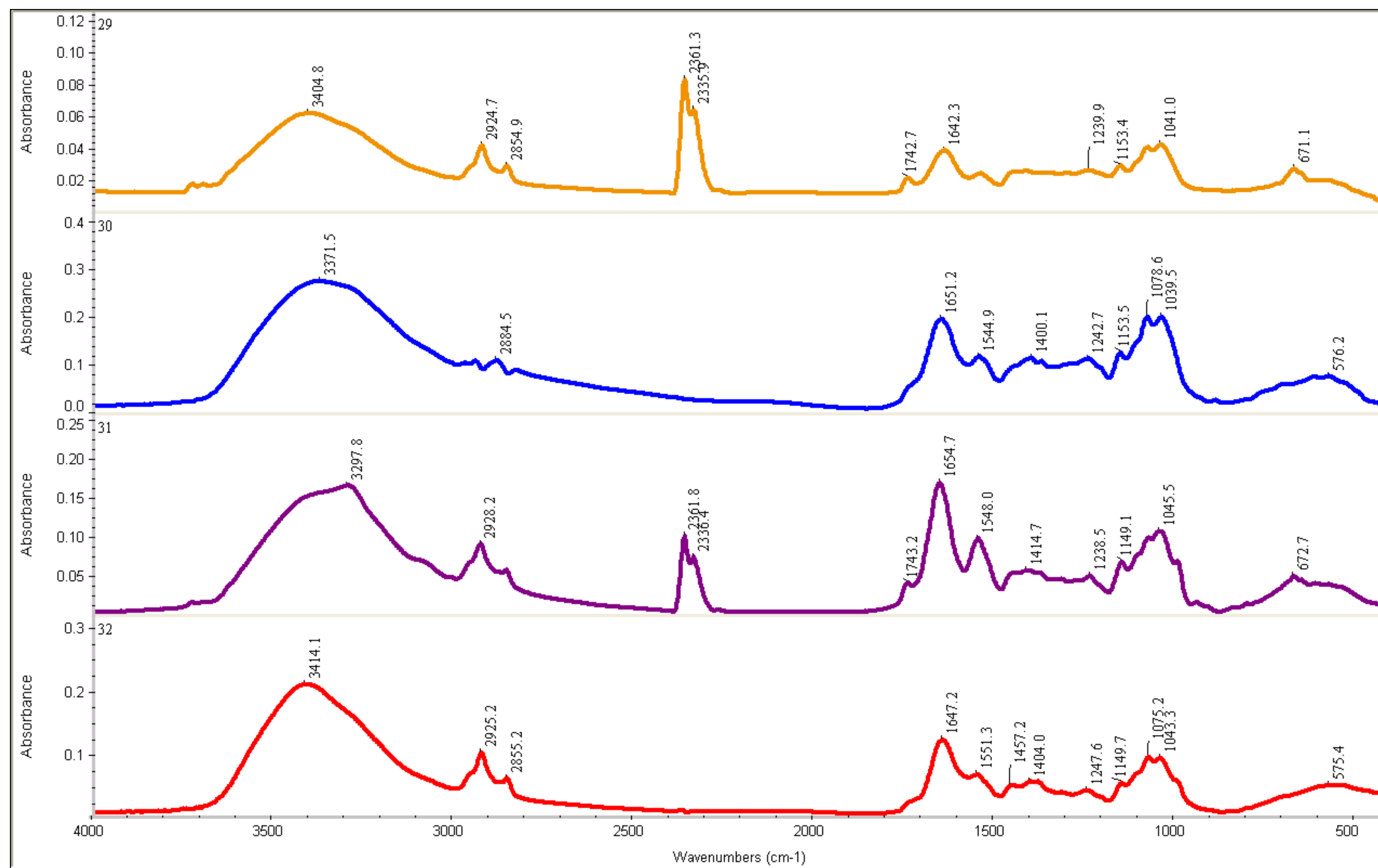


Figure 3.8 FTIR absorbance spectra of guanophilic fungi such as *C. resinae* (29), *C. tenuissimum* isolate VE8 (30), *C. tropicum* (31) and *P. varitii* (32).

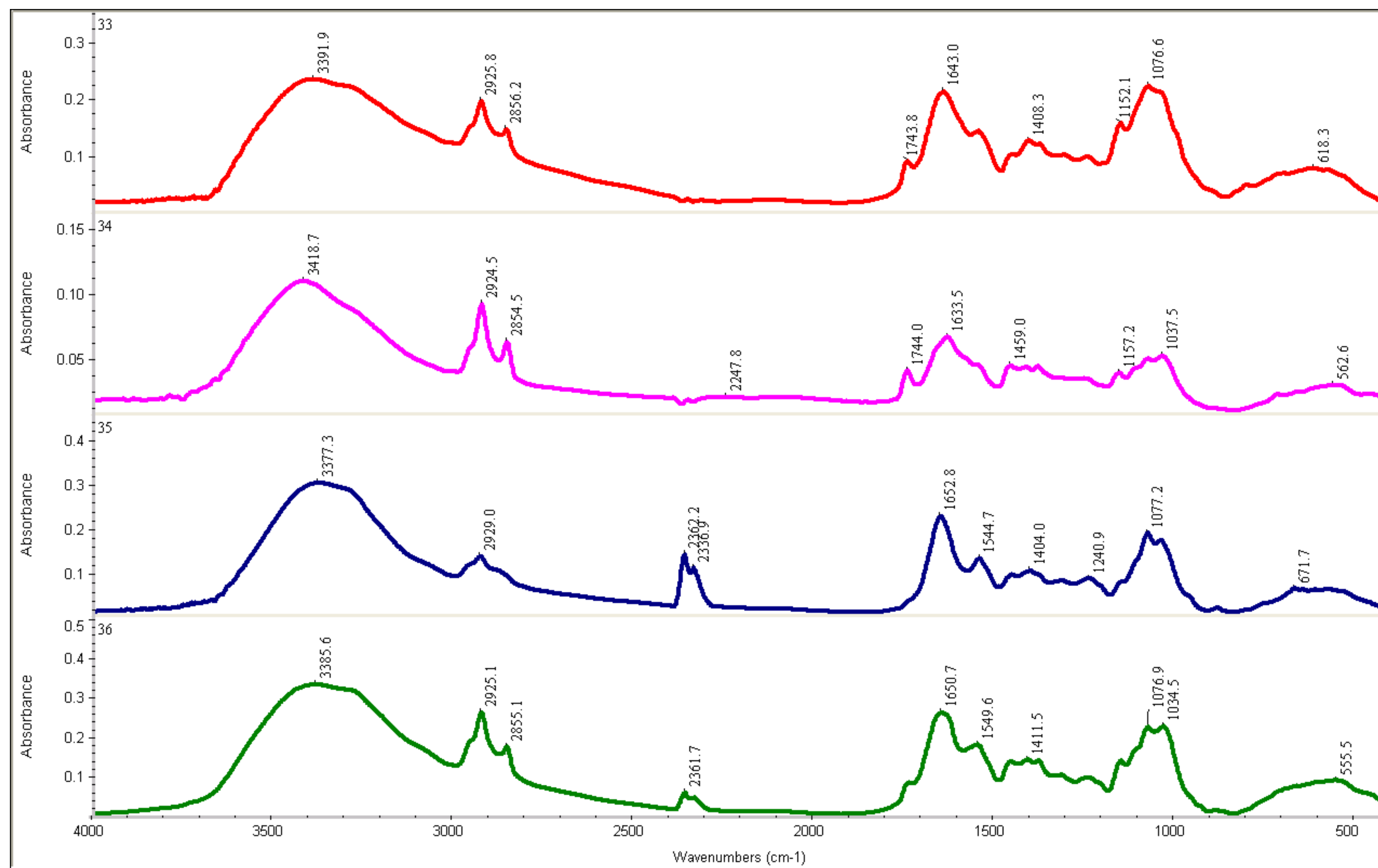


Figure 3.9 FTIR absorbance spectra of guanophilic fungi such as *A. corymbifera* (33), *Cladosporium* sp. VE3 (34), *Davidiella* sp. PKM20 (35) and *Trichocomaceae* sp. PKM21 (36).

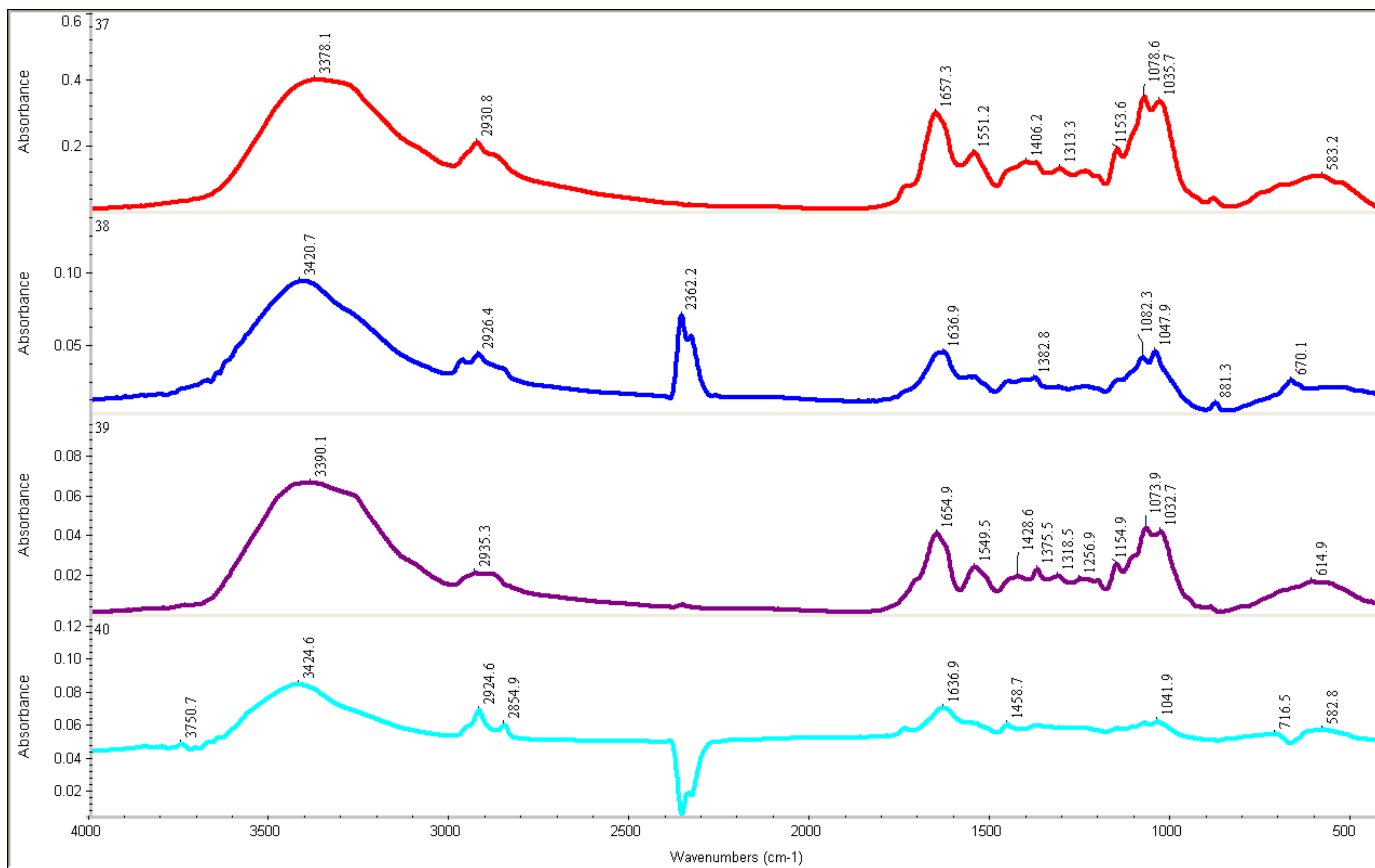


Figure 3.10 FTIR absorbance spectra of guanophilic fungi such as *Periconia* sp.VE2 (37), *M. indicus* isolate BBAU (38), *Mucor* sp. (39) and *Malbranchea* sp. (40).

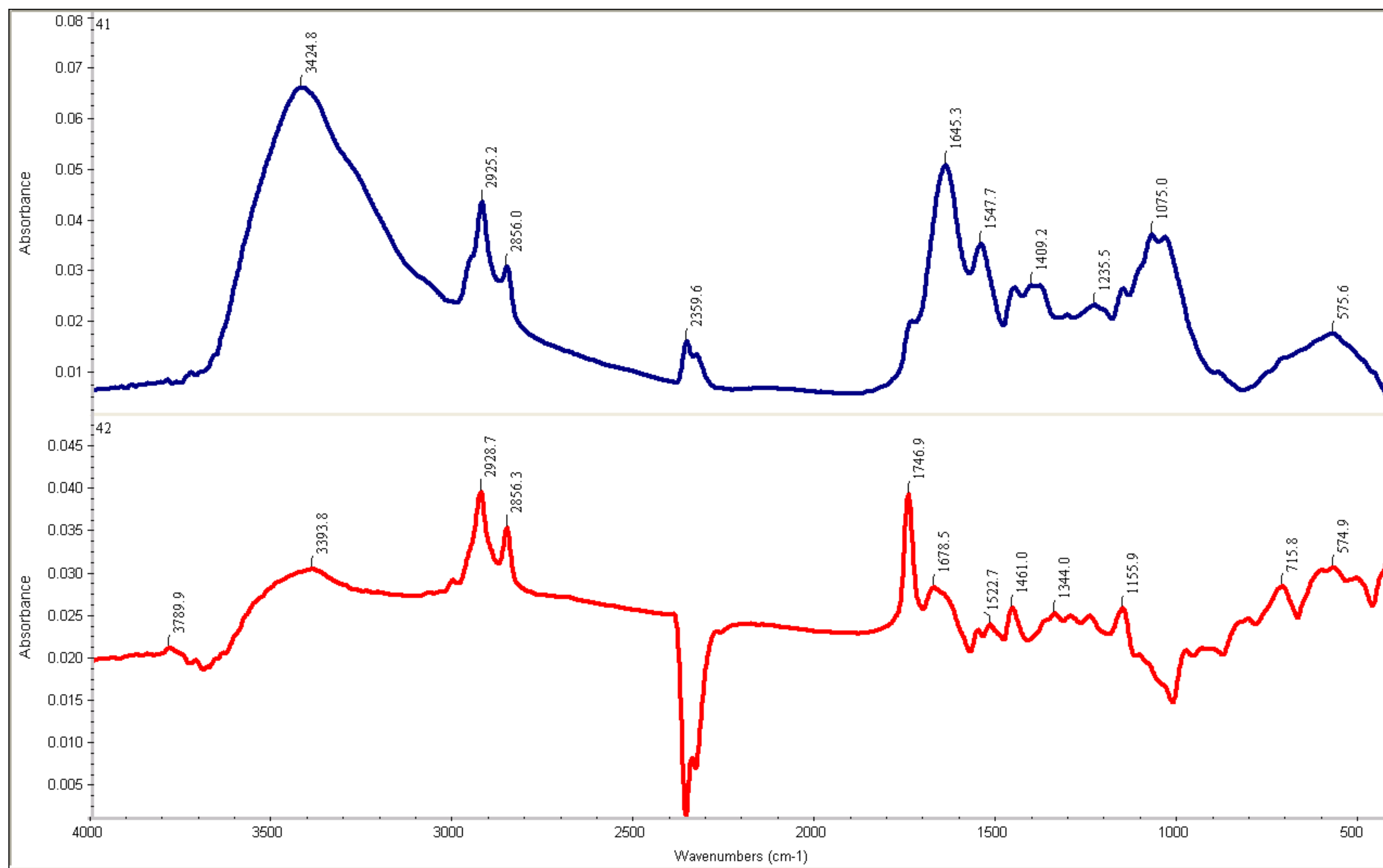


Figure 3.11 FTIR absorbance spectra of guanophilic fungi such as *Trichoderma* sp. (41) and Yeast (42).

Chapter 04:

Diet analysis and diet composition of bat guano

Chapter 04

Diet Composition of Insectivorous Bats

Introduction

Among mammals, bats play unique role in ecological balance, nutrients cycling and redistribution of forests. Insectivorous bats consume a large quantity of insects every night (Whitaker, 1995). Depending on the locations of foraging and roosting habitats, bats potentially could import nutrients among various habitats. The high metabolic rate of insectivorous bats during flight requires large amount of food (Altringham, 1996), that's why they are called voracious feeders of nocturnal insects including many crop and forest pests. Contradictory study results raise the question of feeding whether they select precise insect species as prey or consume solely those species occurring in the greatest abundance remains unclear (Fenton, 1995). Insectivorous bats primarily feed on insects (Adams, 2003), they mainly preys on moths, flies, midges, mosquitoes, mayfly's ground beetles, June bugs, cucumber beetles and other insects that causes massive alleviation to crops. Results from earlier studies also reveal that the foraging time of a bat and the activity period of insect pests consumed by the bat species also coincide and increasing the probability of pest control (Sophia, 2010).

The intensive foraging of insectivorous birds and bats is well known to reduce the density of arboreal herbivorous arthropods, but collateral leaf damage remains limited. Aerial bats reduce herbivore through predation. Exclusion of bats leads to a distinct increase in arthropod herbivory, emphasizing the prominent role of vertebrate predators in controlling arthropods.

Rhinopomatidae (Mouse-tailed bats) is monogenic family and include three species of bats, namely *R. hardwickii* (Lesser mouse-tailed bat), *R. microphyllum* (Greater mouse-tailed bat) and *R. muscatellum* (Small Mouse-tailed bat). In Jordan Valley, northern Israel greater mouse-tailed bat (*R. microphyllum*) feeds on alates of Carpenter ants (*Camponotus* sp.) during nuptial flight of Carpenter ants because in summer, nuptial flight tends to be short in time and assumed to be synchronous across a large area, this fat and protein-rich diet enable greater mouse-tailed bat to lactate during summer, and the large amount of fat that both sexes accumulate may serve as an energy source for their following winter hibernation andpost-hibernation mating in early spring (Levin *et al*., 2008). Ant alates are common food items for many insectivores, including bats (Whitaker, 2004). The rat-tailed bat, *R. microphyllum kinneari* consumedColeoptera, Lepidoptera and Orthopterathroughout the year, while Hymenopterais devoured in winter season, during summer and monsoon months Isoptera is a very preferred diet, Neuroptera and Dictyopteraare consumed in winter and post monsoon seasons, depending upon their availability in the nature (Advani, 1981). The feeding of *R. mirophyllum umkinneari* on some of the most noticeable insect pests of various crops in Rajasthan, such as White grub, Red hairy Caterpillar, many polyphagous grasshoppers, and termites, show that this species is managing the harmful insect population in the natural crop ecosystem, in some stomachs, nymphal stages of the Desert Locust, *Schistocerca gregaria* also recorded (Advani, 1981). Having such an ecologically significant position they play important role as pest-controller.

Vesper bats (family Vespertilionidae), also known as evening bats or common bats, are the largest known family of bats, they are small in size, usually with dull brown fur. *Scotophilus* is commonly called yellow bats, this genus composed of 2 species such as *S. kuhlii* and *heathii*. The average body length is 130 mm and the

weight is about 27 gm. The back is covered with soft, short fur with a hint of brown, and the abdomen is covered with bright yellow fur. *Scotophillus heathii*is a robust bat, the tail is long and muzzle is broad and blunt. The head and back have pale buffy brown hair roots. In some individuals the back is chestnut brown and the belly reddish or golden yellow. *Scotophilus kuhlii* can only be distinguished with certainty from *S. heathii* by its small size. The lesser asiatic yellow bat (*S. kuhlii)* mainly feeds on insects of orders Diptera, Coleoptera and Hymenoptera, the dipterans included representatives of the families Anisopodidae, Chironomidae, Culicidae and Scatophagidae, and coleopterans including representatives of the families Carabidae and Scarabaeidae, while the hymenopterans were represented by Ichneumonidae (Srinivasulu *et al*., 2010).

Megaderma lyra belongs to family Megadermatidae commonly known as Indian false vampire bats. It can be easily distinguished by its large medially fused pinnae and triple lobed nose-leaf. *Megaderma lyra* ranges from Afghanistan to southern China to Pakistan, India, Bangladesh (Bates andHarrison, 1997), Burma, Srilanka (Yapa *et al*., 2005), Thailand and Malaysia. In Indian subcontinent distribution of *M. lyra* in certain region like Central and Western India, Bihar and, Rajasthan (Adavni, 1981), Tamil Nadu (Ramanujam and Verzhutskii, 2004). *Megaderma lyra* comes under heterogeneous group of echolocating bats called gleaners because they prefer to capture prey such as on large insects and small vertebrates such as frogs, mice, fish, and geckoes (Advani, 1981) from ground and water surfaces. Some reports documented that they also feed on small birds (Green, 1907), small bats such as *Pipistrellus mimus* (McCann, 1934; Phillips, 1922) and *Taphozous perforatus* (Prakash, 1959).

Tophozousnudiventris (Naked-rumped tomb bat) is medium sized species, characterized by its naked rump, which contain copious fat reserve, especially in post monsoon period. Male has larger gular sac about 10 mm in breadth. The chin and throat

are essentially naked. They are blackish brown in color and semi-translucent. It has a wide distribution in Africa, ranging from Mauritania to Egypt and Asia.

Family Hipposideridae (Leaf-nosed bat) bats are discerning but opportunistic in feeding their prey insects. The nocturnal foraging behavior, echolocation capability, dentition and flight movement of this bat species are the adaptations that enhance their ability to capture nocturnal prey insects in open space during their foraging flight (Pavey and Burwell, 2001). Bats need elemental nutrient resources based upon which depends on the diversity of the prey species failing to which may lead to compromised reproductive rates (Barclay, 1991). Calcium is indicated to be such an essential factor in the diet of bats; one would predict that females and volant juveniles would seek accessible sources of calcium in the environment (Rick *et al*., 2003).

Bat guano is very important for microflora because it contains all essential chemicals which are necessary for their growth. In many parts of the world, the bat guano is widely used as fertilizer due to the high nitrogen content and bat urine has some nematocidal effects (Keleher and Sara, 1996). Today use of vermicompost to improve soil fertility is very popular but application of bat guano as organic manure (Sridhar *et al*., 2006) is very little known. (Incidents/reports of guano used as fertilizer)

Whitaker (1978) purposed that identification of prey consumed by insectivore's bat, at least, order level possible because most bats do not feed on diverse categories of insects at the solitary period. Thus, an important advantage of analyzing faecal pellets is better easier and precise in the identification of prey subsequently separate items are frequently large and diagnostic (wing and head parts) but relative volumes of various food items not possible.

The present study was aimed to fulfill the lacuna on food and feeding habits of insectivorous bats and also to explore the elements presents in the guano of insectvorous bats.

Materials and methods

Sample collection

The present study was carried out between June 2012 and June 2015. The guano samples of insectivorous bats such as *R. hardwickii*, *R. microphyllum, S. heathii, S. kuhlii,P. coromandra, T. nudiventriss M. lyra and H. fulvus* were collected from their roosting sites by spreading 2 x 2 m polythene sheet beneath the roosting sites. Guano samples were collected in 5 ml sample vials and kept in the refrigerator (at 4 $^{\circ}$C) for further analysis in the laboratory.

Analysis of faecal pellets

Each pellet was dissolved in distilled water and the insect remnants were separated using forceps and magnifying glasses. Recognizable insect body parts were taken out and identified up to order by following identification keys (Richard, 1977).

Sample Preparation for Light Microscopy

Remnants of bat guano were dehydrated from ascending series of alcohol dissolved in triple distilled water i.e. 30%, 50%, 70%, 90% and absolute alcohol. Slides were prepared and examined under the Light Microscope (Olympus CX-40, Olympus, USA). Photographs were taken using Olympus Digital Camera C7070WZ (Olympus, USA).

Sample Preparation for Scanning Electron Microscopy

The insect remnants of bat guano were fixed in 2.5% glutaraldehyde for 2-4 h at 4˚C, washed thrice with phosphate buffer saline (0.1M, pH 7.2) at 15 min interval. Thereafter, post-fixation was done using 1% osmium tetroxide for 2 h at 4 ˚C and washed in 0.1 M phosphate buffer thrice each at 15 min interval at 4˚C. The samples were dehydrated in ascending series of acetone followed by dry acetone. Samples were mountedon the stubs with carbon adhesive tapes. Samples were kept in desiccators overnight and coated with palladium using sputter coater and analysed under Scanning Electron Microscope (JEOL JSM 6490 LV, JEOL, Japan).

Results

Identification of insect remnants in bat guano

A total of eight species of insectivorous bats such as *R. hardwickii*, *R. microphyllum*, *S. heathii*, *S. kuhlii*, *P. coromandra*, *T. nudiventris*, *M. lyra*and *H. fulvus*were found in 15 districts of Uttar Pradesh, India (Table 1.1). The roosts of insectivorous bats were observed in historical monuments, caves, tree cavity, crevices, old abandoned buildings, underground tunnels and temples. The faecal pellets of different insectivorous bats showed that they fed on insects belong to the order Coleoptera, Hymenoptera, Odonata, Hemiptera, Neuroptera, Lepidoptera and Diptera (Table 4.1).

The guano samples of *R. hardwickii*were collected from Allahabad, Varanasi, Jaunpur, Mirzapur and Pratapgarh districts of Uttar Pradesh (Table 1.1). The guano samples of *R. hardwickii*had the body parts of Coleoptera, Hymenoptera, Odonata and Neuroptera insect (Table 4.1). The undigested leg parts of Hymenoptera (Fig. 4.1 A, B and C), Coleoptera (Fig. 4.1 D), Odonata (Fig. 4.1 F and G) and Orthoptera (Fig. 4.1 H

and I) were observed in the guano of *R. hardwickii*. Further, the guano of *R. hardwickii* had wings of Neuroptera (Fig. 4.1 J and K) and Elytra of Coleoptera (Fig. 4.1 E).

The roost of *R. microphyllum* was only found at Chunar Fort, Mirzapur (Table 1) and the guano samples had wings of Hymenoptera (Fig. 4.1 L and Fig. 4.1 M), legs of Diptera (Fig. 4.1 P), Coleoptera (Fig. 4.1 N, Fig. 4.2 F).

Guano samples of *S. heathi i*were collected from Purwa, Unnao, Ayodhya, Faizabad and Hardoi districts (Table 1.1). The remnants belong to two insect orders such as Hymenoptera and Coleoptera were found in the guano of *S. heathii* (Table 2). A complete beetle (Fig. 4.2 B), wings of Hymenoptera (Fig. 4.1 S), elytra (Fig. 4.1 T) and antenna (Fig. 4.2 C) of Coleoptera were observed in the guano of *S. heathii.*

Guano of *S. kuhlii* was collected from Jaunpur, Hardoi and Lucknow districts (Table 1). Insect body parts belong to orders Hymenoptera, Coleoptera, Diptera, Odonata, Orthoptera and Lepidoptera were recorded from the guano samples of *S. kuhlii* (Table 4.1). In addition, the legs of Hymenoptera (Fig. 4.1U, Fig. 4.2 D and G), Orthoptera (Fig. 4.2 S) and Lepidoptera (Fig. 4.2 T), Diptera (Fig. 4.2 Q - Q) and wings of Odonata (Fig. 4.2 R) were observed.

Pipistrellus coromandra was collected from Chunar fort, Mirzapur district and found the antennae of coleopteran insects (Fig. 4.2 U and Fig. 4.2 V).

The guano samples of *T. nudiventris* were collected from Jhansi and Hardoi. The food remnants belong to Coleoptera, Orthoptera, Hymenoptera were observed from the guano of *T. nudiventris* (Table 4.1). The remnants include abdominal segments (Fig. 4.2 X) and legs (Fig. 4.2 Y, Z) of Coleoptera, legs (Fig. 4.1V, 4.2 AA) and wings (Fig. 4.2 AB) of Orthoptera and legs (Fig. 4.2 AC, AD) and wings (Fig. 4.1 W) of Hymenoptera were observed.

The guano samples of *M. lyra*were collected from Purwa, Unnao, Hardoi, Bahraich, Sultanpur and Mirzapur (Table 1.1). In the guano of *M. lyra*, the remnants belong toorders Coleoptera, Hymenoptera andOrthoptera were observed. The antenna (Fig. 4.1 X – Z, Fig. 4.2 AE) of Coleoptera and mandible (Fig. 4.1 AA) of Hymenoptera (Fig. 4.1 AE) were observed.

The guano of *H. fulvus*was collected from Bara Imambara, Lucknow (Table 4.1). The remnants include legs of Hymenoptera (Fig. 4.2 AG - AJ), Coleoptera (Fig. 4.2 AK) and Orthoptera (Fig. 4.2 AO and AP), wings (Fig. 4.2 AK), antenna (Fig. 4.2 AL) and elytra (Fig. 4.2 AM) of Coleoptera were observed in the guano of *H. fulvus*.

Elemental analysis of bat guano

A total of 15 elements such as Aluminum (Al), Boron (Br), Calcium (Ca), Copper (Cu), Chlorine (Cl), Iron (Fe), Potassium (K), Manganese (Mn), Magnesium (Mg), Sodium (Na), Phosphorous (P), Sulpher (S), Titanium (Ti), Zirconium (Zr) and Zinc (Zn) observed in the guano samples of bats (Table 4.2 and Fig. 4.3 – 4.10).

The results of the present study reveal the presence of a wide range of mineral constituents in bat guano. Out of 21 point analysis the element K (21) was most frequently occurred and Mn / Zn were observed once followed by Br (2) and Cu (2). The highest percentage of P (14.52 ± 16.61) and minimum percentage of Mn 0.41 ± 0.001 was observed in the guano samples of *R. hardwickii*. The highest quantity of Zr (29.26 ± 9.04) was observed in the guano sample of *R. microphyllum*.

Four samples of *S. heathii* was found in the study area, in total 12 point analysis most frequent occurring elements were Mg and Na and least observed elements were Ti. The highest percentages of K (16.39 ± 14.30) and least percentage of Ti (0.95 ± 0.41) were observed in the guano samples of *S. heathii*.

In the guano sample of *S. kuhlii*S and Cl were recorded maximum (14 times) occurring elements and Zr and Zn were minimum (02 times). Elements such as S and Cl were commonly observed in the guano of *S. kuhlii*, however the quantity of Zr (16.32 ± 2.11) was higher.

Single samples of *P. coromandra* and *H. fulvus* each were recorded in study sites. In the guano of *P. coromandra* K was highest 16.27 ± 13.75 and Zn was in very least amount 2.29 ± 1.34. Ca 67.80 ± 17.71 was highest, and S was minimum 2.98 ± 5.92 in *H. fulvus* guano sample. In the guano of *T. nudiventris* guano, K was maximum occurring elements, also highest in percentage 19.72 ± 18.21, Ti was very rare and least in percentage 2.29 ± 0.00. *Megaderma lyra* were recorded five places in present study most frequent occurring (15 times) elements K (15.92 ± 13.50) followed by S (8.76 ± 8.27) and Cl (7.97 ± 12.18) and least observed (single times) Zr 2.66 ± 0.00.

Discussion

The results of present study revealed that the insectivorous bats commonly feed on the insects of seven insect orders such as Coleoptera, Hymenoptera, Odonata, Hemiptera, Neuroptera, Lepidoptera and Diptera. According to Oliveira (2005), the Coleopterans, Lepidopterans, Homopterans and Hemipterans are among the major pests in farms.

The remnants of coleopteran insects were observed in the guano samples of all bat species investigated in this study. The body parts of Coleopteran were observed in all the species of insectivorous bats guano isolated. Hymenoptera was also recorded in all bat species except *P. coromandra.* Maximum insects order represented in the guano of *S. kuhlii,* out of eight insect orders six insect order Coleoptera, Lepidoptera, Diptera, Hymenoptera, Orthoptera and Odonata were found. An earlier report by Srinivasulu *et*

al., 2010 has shown three insect orders Diptera, Coleoptera and Hymenoptera in the diet of *S. kuhlii*. In our study *P. coromandra* guano only represented Coleopteran insect order. Faecal matter from *T. nudiventriss*, *M. lyra* and *H. fulvus* revealed only three insect orders Coleoptera, Hymenoptera and Orthoptera.

Our results lead to the conclusion that various species of bats are selective to certain orders of insects which may lead to the biological control of the target insect pest in agriculture. The use of biological pest management is more important in current scenario as continuous uncontrolled means of chemical control has led to calamitous consequences. Thus, our study supports the need to conserve the bats in their natural habitat.

The elements such as Ca, K, Mg, S, P, Cl, Br, Cu, Fe, Mn and Zn found in the guano are essential for the growth of plants (Elizabeth *et al*., 2009). The six macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) were abundant in the bat guano. The abundance of macronutrients in bat guano suggests its suitability as bio-fertilizer. It has been already reported that guano of insectivorous *Hipposideros speoris* amended in soil at a ratio of 1:20 which serves as a good fertilizer (Sridhar *et al*., 2006). Bat guano is reported to contain all the macro and micro nutrients required by plants and besides being a good fertilizer it also serves as soil builder, nematicide (Keleher and Sara, 1996), compost activator and used to improve the quality of poor roughage (Paul and Sagamiko, 2008). Insects are inadequate source of calcium and thus calcium was limited in the guano of insectivorous bats. Presence of high level potassium in the guano of insectivorous bats also suggests that they consume a large amount of Lepidopteron (Studier *et al*., 1994). Calcium play a multifunctional nutrient role in the physiology of crop plants andsoluble form influences availability and uptake, soluble calcium source also increases uptake of

nitrogen from urea containing fertilizer as a calcium nitrate (Easterwood, 2002). Deficiency of calcium includes death of growing points, abnormally dark green foliage, premature shedding of blossoms, weakened stems and buds (Easterwood, 2002). Use of calcium increase the strength and thickness of cell wall by forming cross-links within the pectin polysaccharide matrix and provide structural integrity of stems that hold flowers and fruit, as well as the quality of the fruit produced, is strongly coupled to calcium availability (Easterwood, 2002).

Boron is only found in the guano of *R. hardwickii*. Boron is an essential element for synthesis of plant hormones Serdar *et al*., 2011).Role of boron at molecular level is lignin formation of cell wall synthesis, cell division, differentiation, membrane functioning, root elongation and salt absorption (Marschner, 1995; Simmons, 1998; Anonymous, 2007). The utmost essential roles of boron in plants are believed to be its structural role in cell wall growth and motivation or inhibition of precise metabolism pathways (Ahmad *et al*., 2009). For the growth and development of species of marine algal flagellates, vascular plants and diatoms boron is an essential micronutrient (Loomis and Durst, 1992). Leguminous plants, as well as cyanobacteria require B for N2 fixation, as B plays a major role in nitrogen assimilation (Brown andShelp, 1997). Boron its deficiency is recognised by word wide more extensive than any other micronutrient of plants (Gupta, 1979, Reisenauer *et al*., 1973).

Chlorine was found in all most all bat guano, the requirement of chlorine is very minimal but very essential for higher plants (Marschner, 1995). Deficiency of chlorine is very rare in nature but high concentration in tissue can be toxic to crop plants, and may restrict the agriculture of saline regions (Xu *et al*., 2000).

Sodium and Potassium found in the guano of insectivorous bats, Sodium is chemically alike potassium and likely enters plants generally via non-selective cation transporters, particulary potassium channels (Davenport and Tester, 2000, Demidchik, 2002), and those plants which C4 or CAM photosynthetic pathways (Ohnishi *et al.*, 1990) sodium is essential to fix atmospheric carbon for photosynthesis (Elizabeth *et al.*, 2009).

Magnesium (Mg) was found in all the guano sample of insectivorous bat except *R. microphyllum* and their percentage was also sufficient, it used as a macronutrient, important for all cereals on high pH mineral (alkaline) and organic soils. Enzyme systems involved with carbohydrate and nitrogen metabolism.

Zinc (Zn) was found only in three bat guano *R. hardwickii*, *S. kuhlii* and *P. coromrndra*, it is very essential in corn and bean production. Deficiencies usually occur on eroded soils low in organic matter with high pH and necessary for sugar regulation and enzymes that control plant growth (Alberta report for all above).

The study also provides a comparative view on varying amounts of elements in the guano of different bat species play a vital role in the growth of plants. Along with its higher fertilizing value, use of guano as a fertilizer is also becoming popular like other studies on the potential of using nonconventional organic manures (Kale *et al.*, 1992; Ashwini and Sridhar, 2002, 2006) in agriculture. It is known fact that guano varying in nitrogen and phosphorus content lead to the differential growth of plant parts. It prompted a novel suggestion to use the guano from selected groups of bats to be used as fertilizer in selective crops to enhance the production and quality.

Table 4.1 Remenanat of various insects orders found in the guano of insectivorous bats.

Bat sp.	**Insect Orders**							
	Coleoptera	**Lepidoptera**	**Diptera**	**Hymenoptera**	**Orthoptera**	**Neuroptera**	**Hemiptera**	**Odonata**
R. hardwickii	+	-	-	+	+	+	-	+
R. microphyllum	+	-	+	+	+	-	-	+
S. heathii	+	-	-	+	-	-	-	-
S. kuhlii	+	+	+	+	+	-	-	+
P. coromandra	+	-	-	-	-	-	-	-
T. nudiventriss	+	-	-	+	+	-	-	-
M. lyra	+	-	-	+	+	-	-	-
H. fulvus	+	-	-	+	+	-	-	-

Table 4.2 Percent elemental share in the guano of different species of insectivorous bats. Values are given as mean ± SD. Sample size is given in parenthesis.

Sp./Elements	*R. hardwickii* (n = 07)	*R. microphyllum* (n = 01)	*S. heathii* (n = 04)	*S. kuhlii* (n = 05)	*P. coromandra* (n = 01)	*T. nudiventris* (n = 03)	*M. lyra* (n = 05)	*H. fulvus* (n = 01)
Al	11.13 ± 12.67 (11)		12.11 ± 13.51 (9)	5.14 ± 5.71 (12)	5.50 ± 6.36 (2)	11.73 ± 11.27 (8)	9.54 ± 6.77 (10)	2.28 ± 3.08 (3)
Br	5.20 ± 2.34 (2)	-	-	-	-	-	-	-
Ca	6.93 ± 4.61 (12)	-	11.58 ± 15.19 (7)	8.73 ± 12.21 (9)	9.42 ± 9.64 (3)	14.78 ± 23.20 (7)	13.84 ± 14.96 (14)	67.80 ± 17.71(3)
Cu	1.78 ± 0.77 (2)	-	-	-	-	-	-	-
Cl	5.84 ± 7.52 (17)	23.93 ± 40.79 (3)	9.85 ± 8.81 (8)	4.48 ± 5.34 (14)	5.08 ± 6.70 (2)	13.12 ± 13.28 (5)	7.97 ± 12.18 (15)	3.57 ± 14.20 (3)
Fe	8.44 ± 8.90 (17)	-	12.00 ± 7.89 (8)	8.00 ± 10.96 (10)	7.93 ± 9.28 (3)	9.04 ± 12.67 (6)	12.47 ± 16.20 (10)	4.65 ± 14.53 (3)
K	11.95 ± 11.84 (21)	21.93 ± 34.64 (3)	16.39 ± 14.30 (110	16.63 ± 16.78 (14)	16.27 ± 13.75 (3)	19.72 ± 18.21 (9)	15.92 ± 13.50 (15)	9.42 ± 31.36 (3)
Mn	0.41 ± 0.00 (1)	-	1.43 ± 0.00 (10	-	-	-	0.70 ± 0.00 (1)	-
Mg	6.73 ± 7.64 (17	-	6.98 ± 8.45 (11)	5.54 ± 4.57 (13)	6.06 ± 5.86 (3)	4.98 ± 4.11 (7)	8.09 ± 8.10 (13)	1.65 ± 1.08
Na	3.72 ± 2.61 (13)	7.73 ± 1.67 (2)	5.07 ± 3.68 (11)	4.04 ± 4.31 (10)	4.68 ± 5.03 (2)	3.32 ± 2.80 (5)	4.39 ± 4.21	2.25 ± 4.06 (3)
P	14.52 ± 16.61(13)	-	12.05 ± 16.77 (8)	11.30 ± 12.31 (11)	11.71 ± 10.03 (2)	7.24 ± 8.25 (8)	14.97 ± 15.60 (13)	5.41 ± 8.08 (2)
S	11.29 ± 13.95 (20)	17.15 ± 13.86 (3)	11.60 ± 10.97 (9)	10.07 ± 7.78 (14)	10.09 ± 7.27 (3)	9.04 ± 6.22 (6)	8.76 ± 8.27 (15)	2.98 ± 5.92 (3)
Ti	1.62 ± 1.81 (6)	-	0.95 ± 0.41 (4)	7.37 ± 16.27 (5)	9.56 ± 13.65	2.29 ± 0.00 (1)	0.71 ± 0.22 (3)	-
Zr	8.58 ± 8.73 (6)	29.26 ± 9.04 (3)	-	16.32 ± 2.11 (2)	11.41 ± 11.08 (1)	4.73 ± 0.00 (1)	2.66 ± 0.00 (1)	-
Zn	1.88 ± 0.00 (1)	-	-	2.39 ± 1.66 (2)	2.29 ± 1.34 (1)	-	-	-

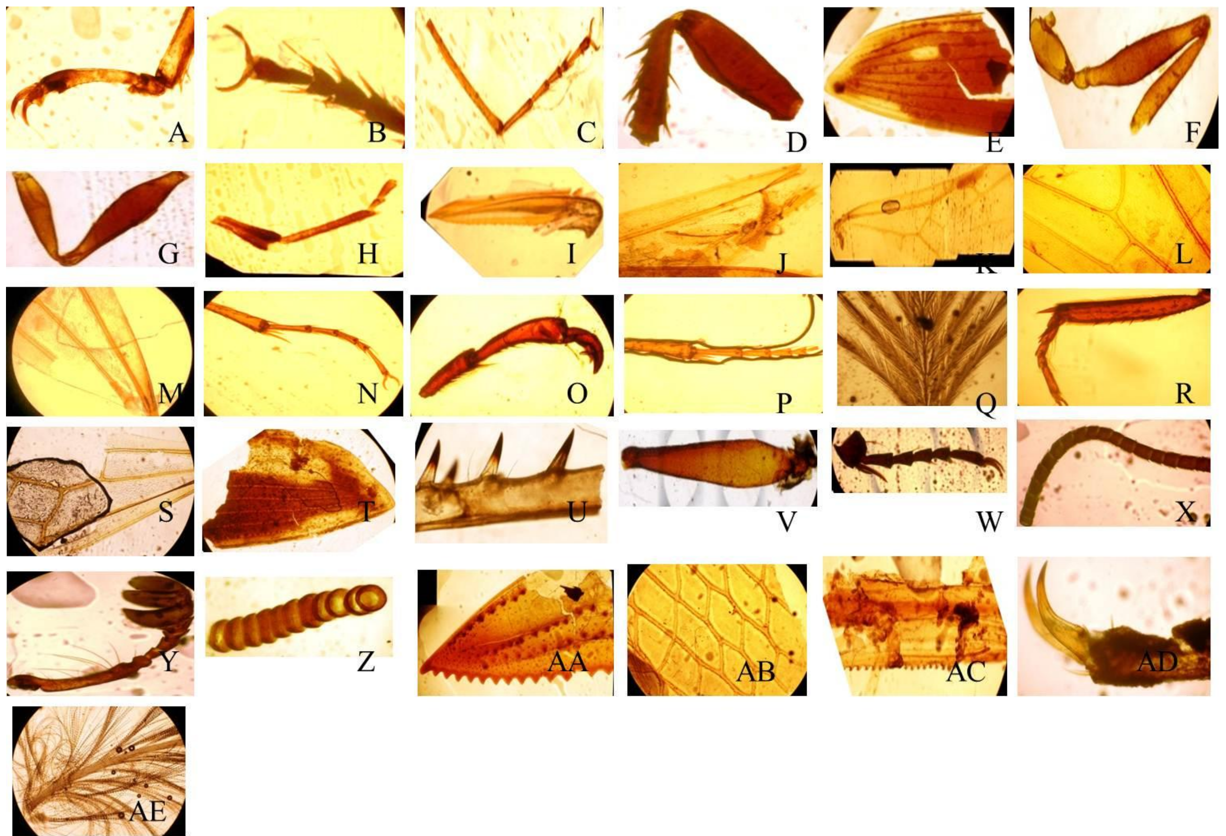


Figure 4.1 Light microscope images of food remnants: Legs of Hymenoptera (A, B and C), Coleoptera (D), elytra of Coleoptera (E), Odonata (F and G) and Orthoptera (H and I), wing of Neuroptera (J and K), wing of Hymenoptera (L and M), Coleoptera (N), leg of Orthoptera (O), legs of Diptera (P), leg of Odonata (Q), feather of a bird (R), Wing of Hymenoptera (S), elytra of Coleoptera (T), legs of Hymenoptera (U), Orthoptera legs (V) Hymenopteran wing (W), Coleopteran antenna (X - Z), mandible of ant (AA) and feather (AE).

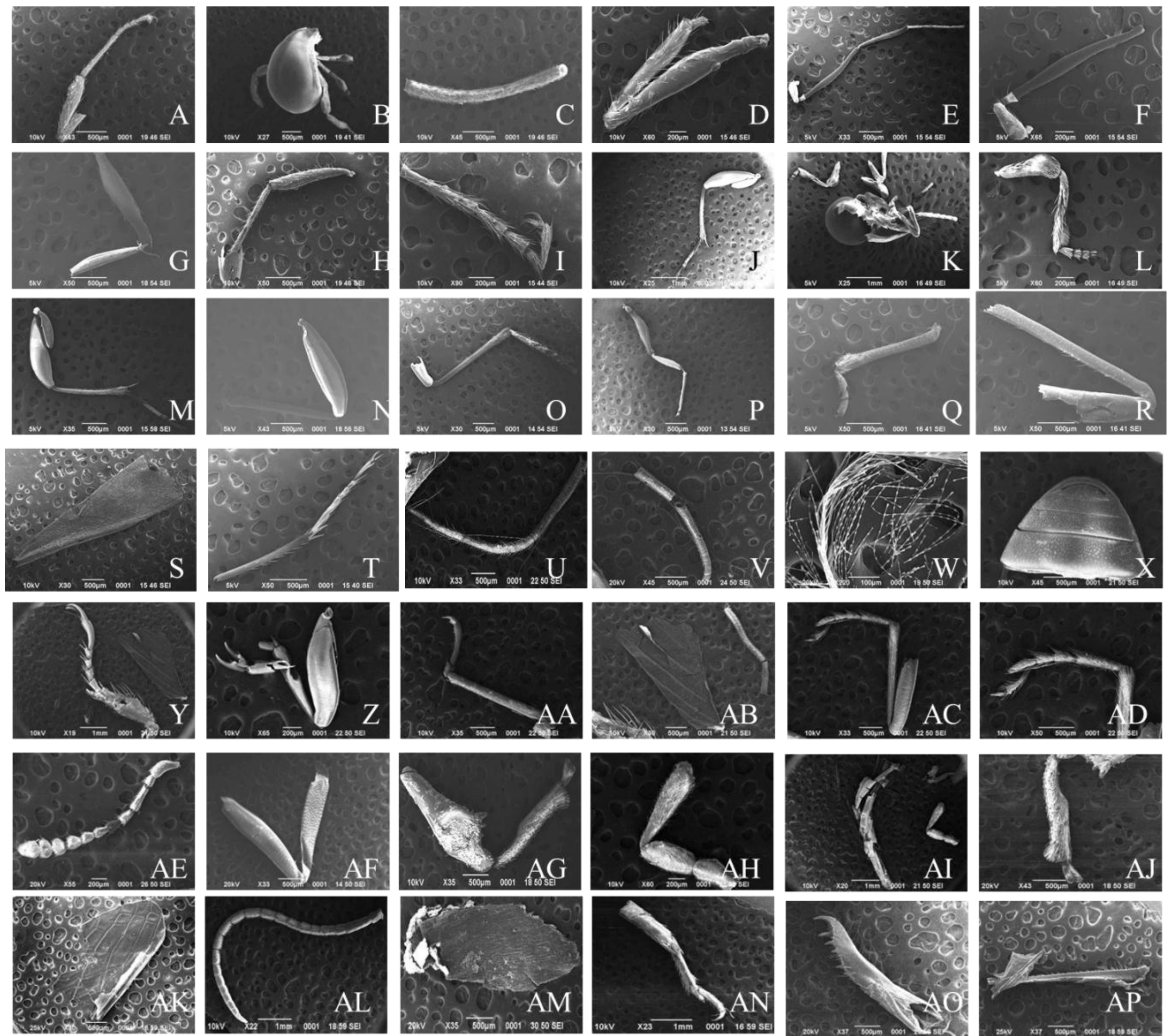


Figure 4.2 Scanning Electron Microscope images of food remnants: Leg of Hymenoptera (A), beetle (B), antenna of Coleoptera (C), legs of Hymenoptera (D and E), leg of Coleoptera (F), leg of Hymenoptera (G), leg of Coleoptera (H – J), whole body of Coleoptera (K), leg of Coleoptera (L – N), leg of Diptera (O – Q), wing of Odonata (R), leg of Orthoptera (S), leg of Lepidoptera (T), antenna of Coleoptera (U – V), fearher of Bird (W), feather of bird (W), Coleoptera abdominal (X) and legs Coleoptera (Y, and Z), Orthoptera legs (AA), Orthoptera wing (AB), Hymenopteran legs (AC and AD), Coleopteran antenna (AE), legs of Hymenoptera (AG - AJ), Coleoptera wing (AK), Coleoptera antenna (AL), Coleoptera elytra (AM), Coleoptera (AN), Orthoptera (AO and AP).

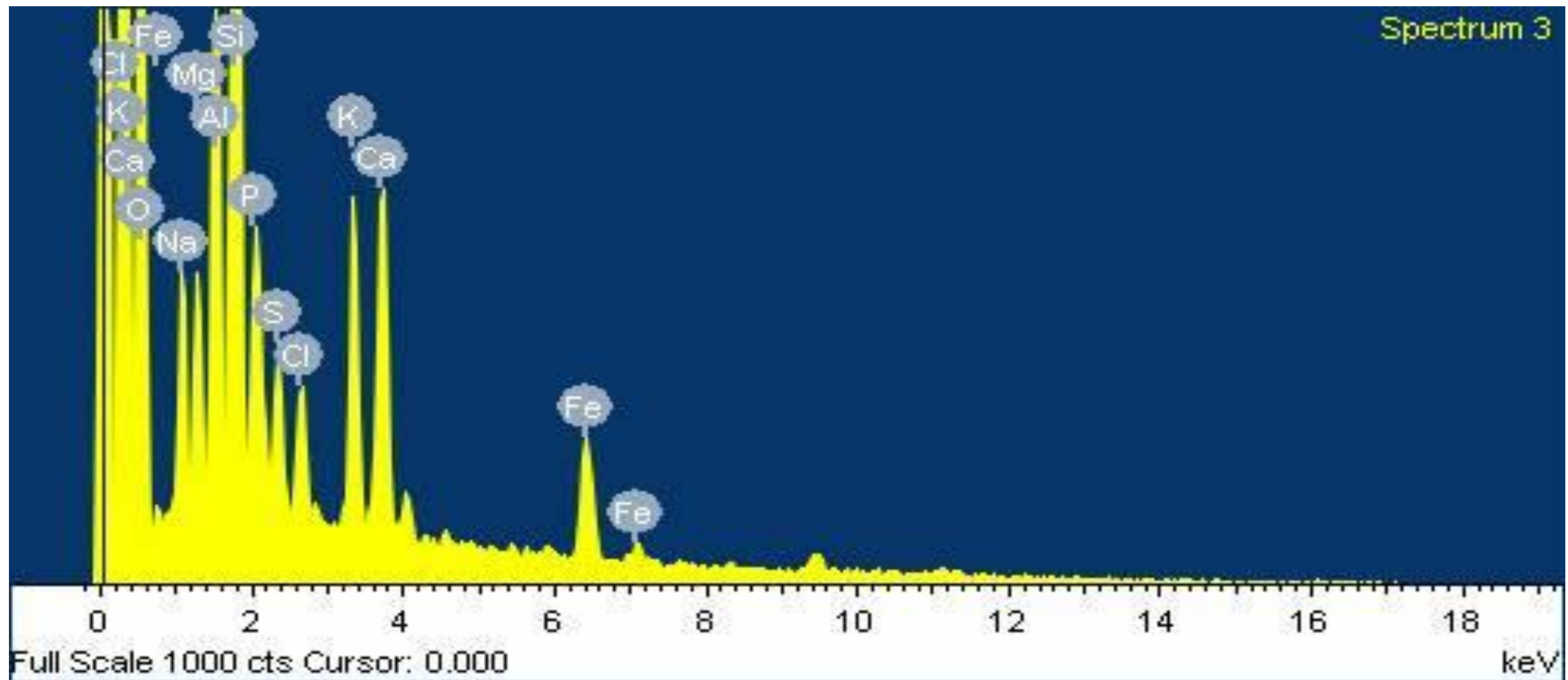


Figure 4.3 SEM-EDS spectra of *R. hardwickii* guano samples shows the presence of elements such as Cl, K, Ca, Na, S, Fe, Mg, Al and P.

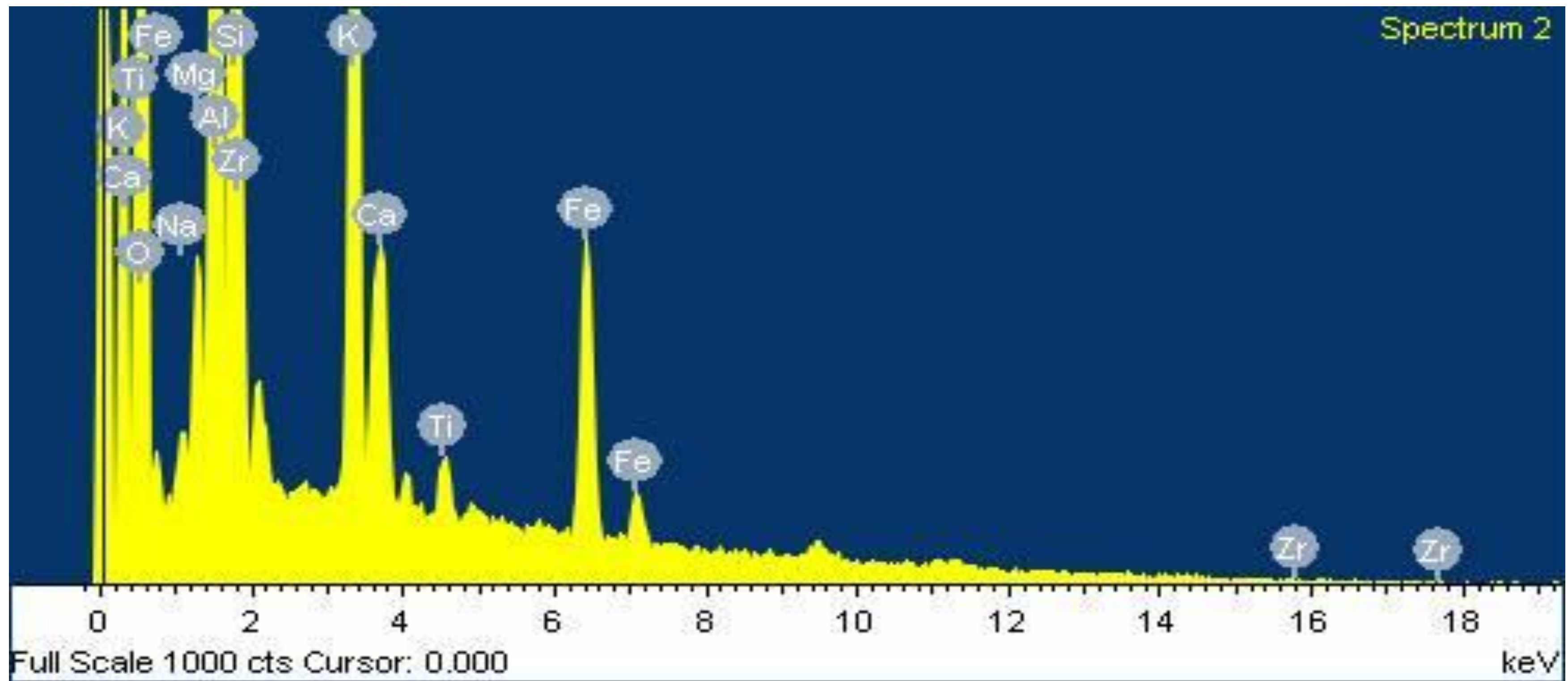


Figure 4.4 SEM-EDS spectra of *R. microphyllum* guano samples shows the presence of elements such as Fe, K, Ca, Al, Mg, Na, S, Zr, Tnd Ti.

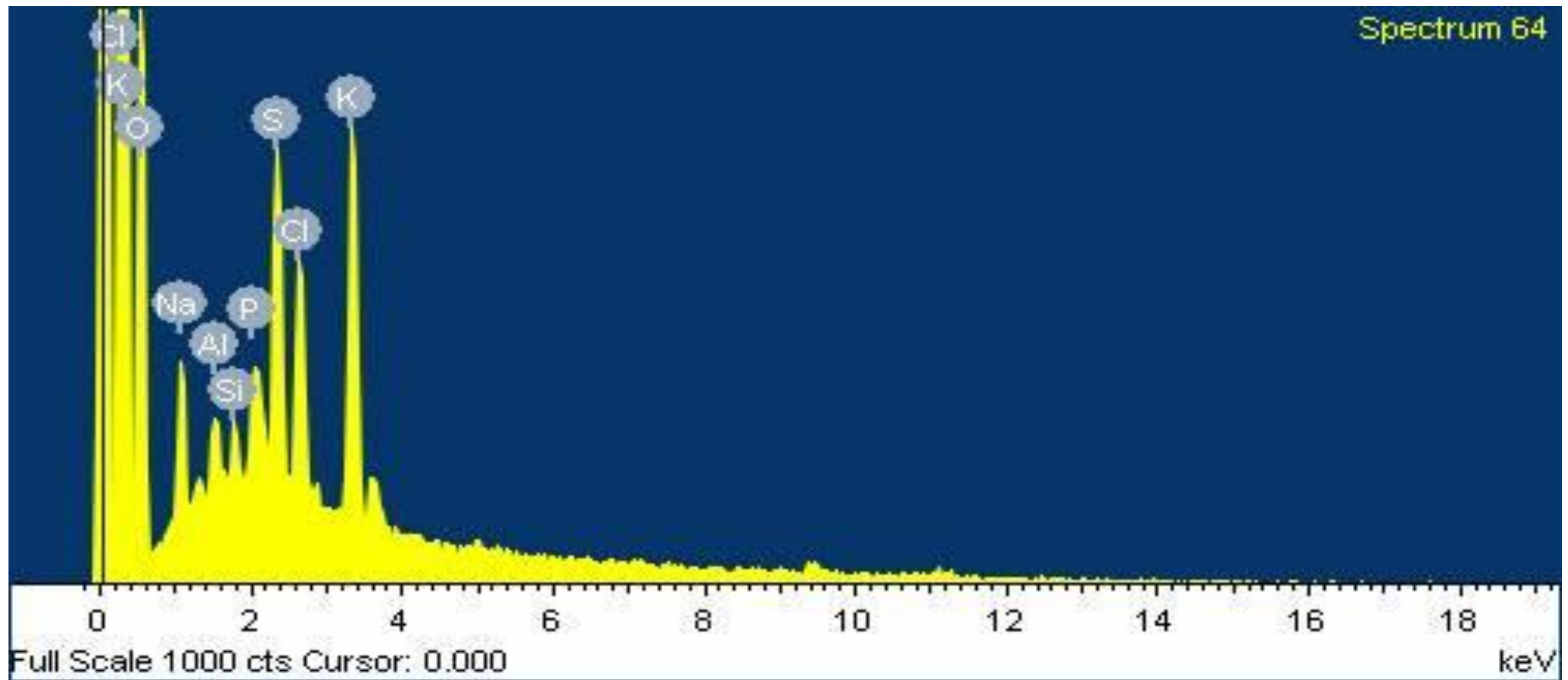


Figure 4.5 SEM-EDS spectra of *S. heathii* guano samples show the presence of elements such as Cl, K, Na, S, Al and P.

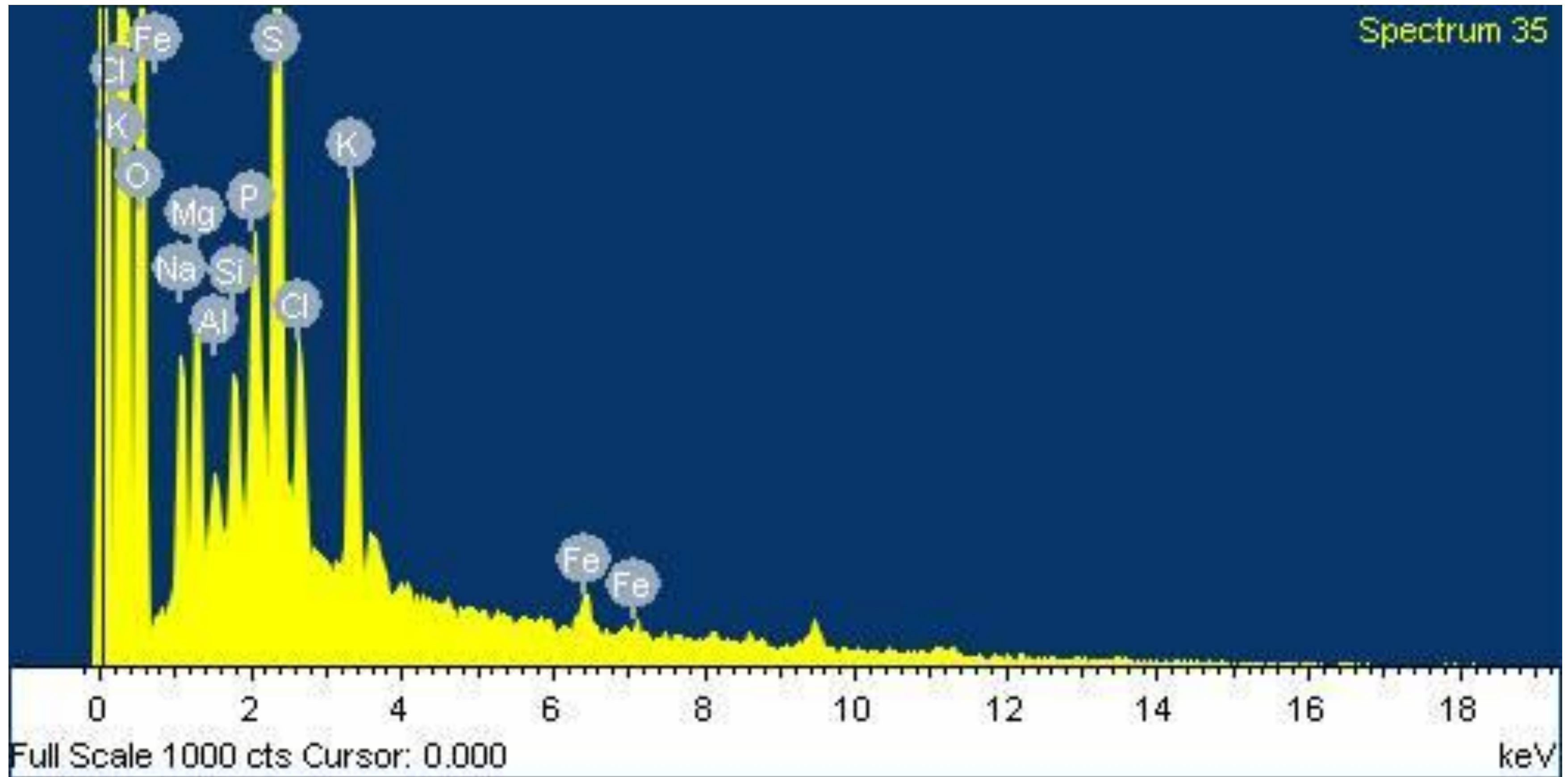


Figure 4.6 SEM-EDS spectra of *S. kuhlii* guano samples show the presence of elements such as Cl, K, Na, S, Fe, Mg, Al and P.

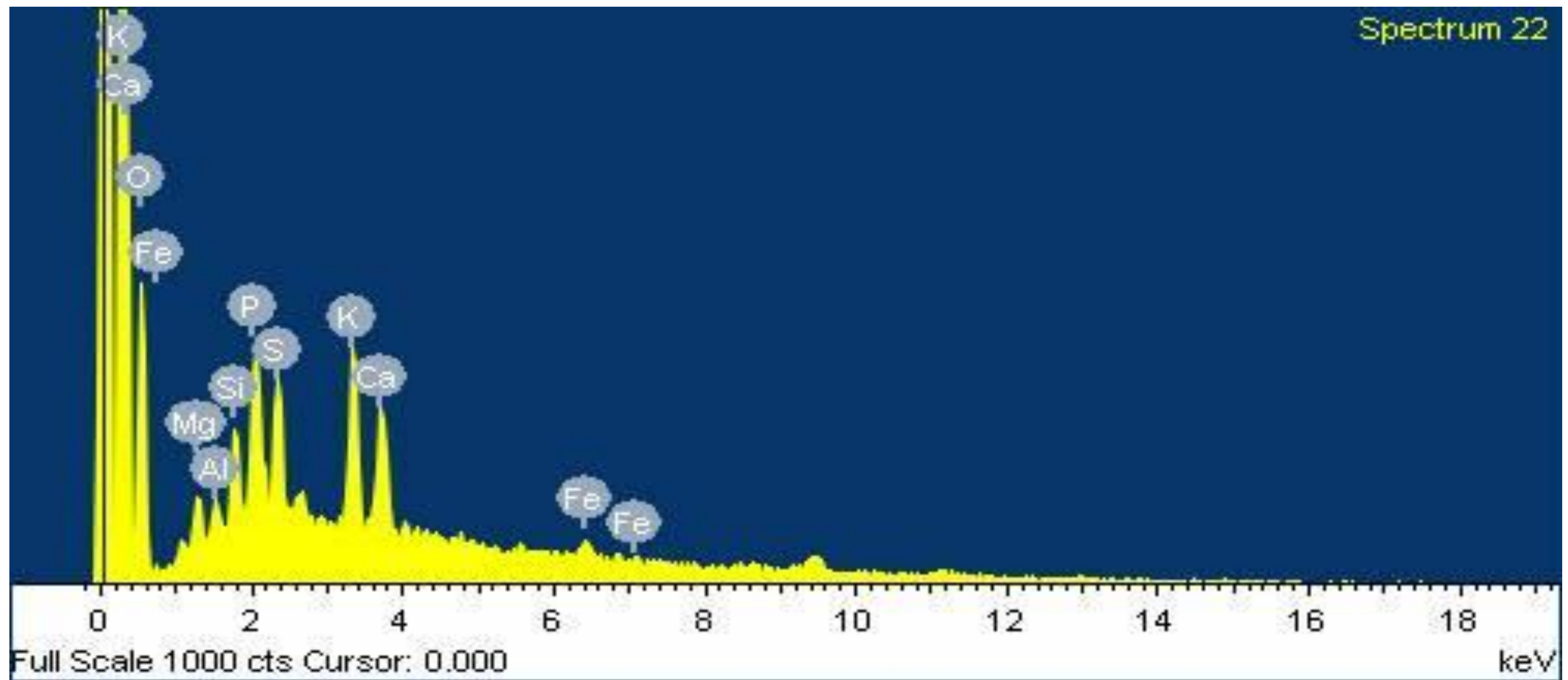


Figure 4.7 SEM-EDS spectra of *P. coromandra* guano samples show the presence of elements such as K, Ca, Na, S, Fe, Mg, Al and P.

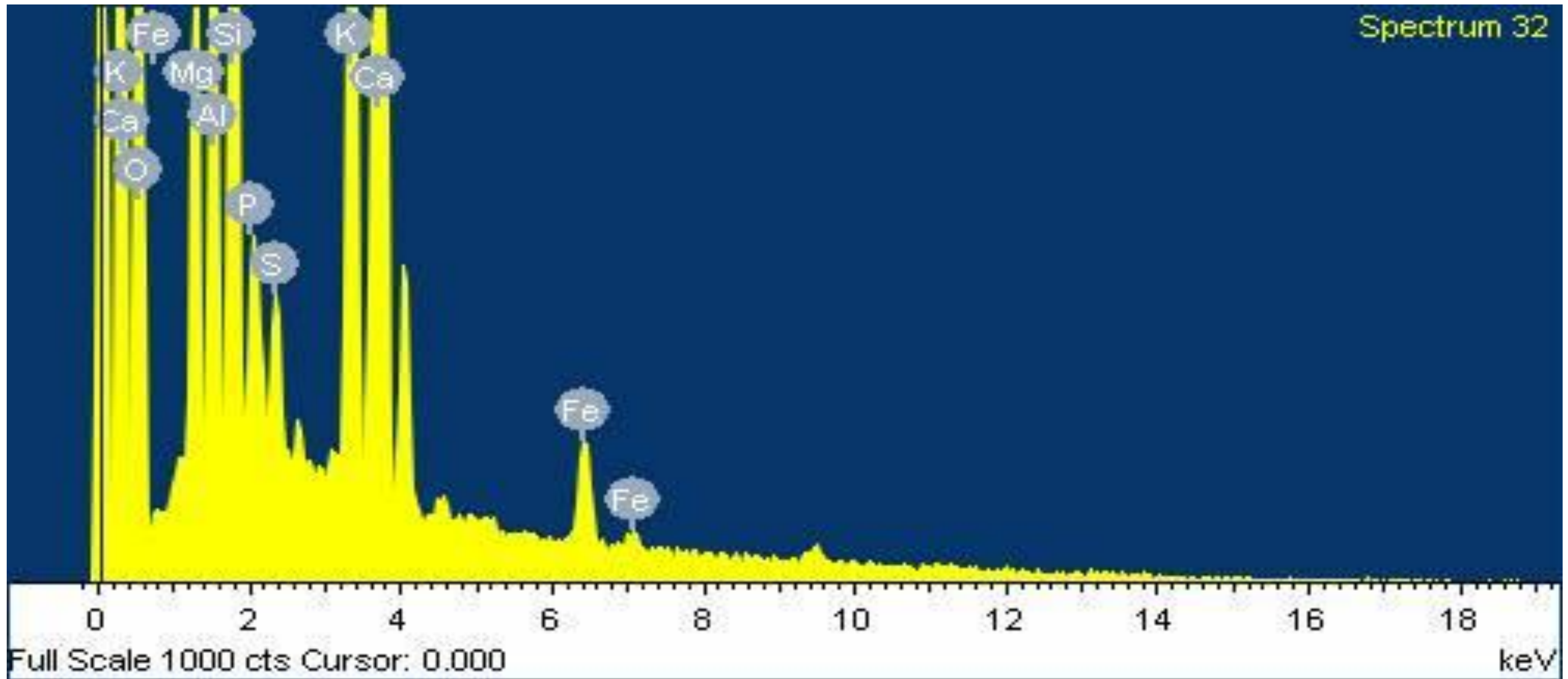


Figure 4.8 SEM-EDS spectra of *T. nudiventriss* guano samples shows the presence of elements such as K, Ca, Na, S, Fe, Mg, Al and P.

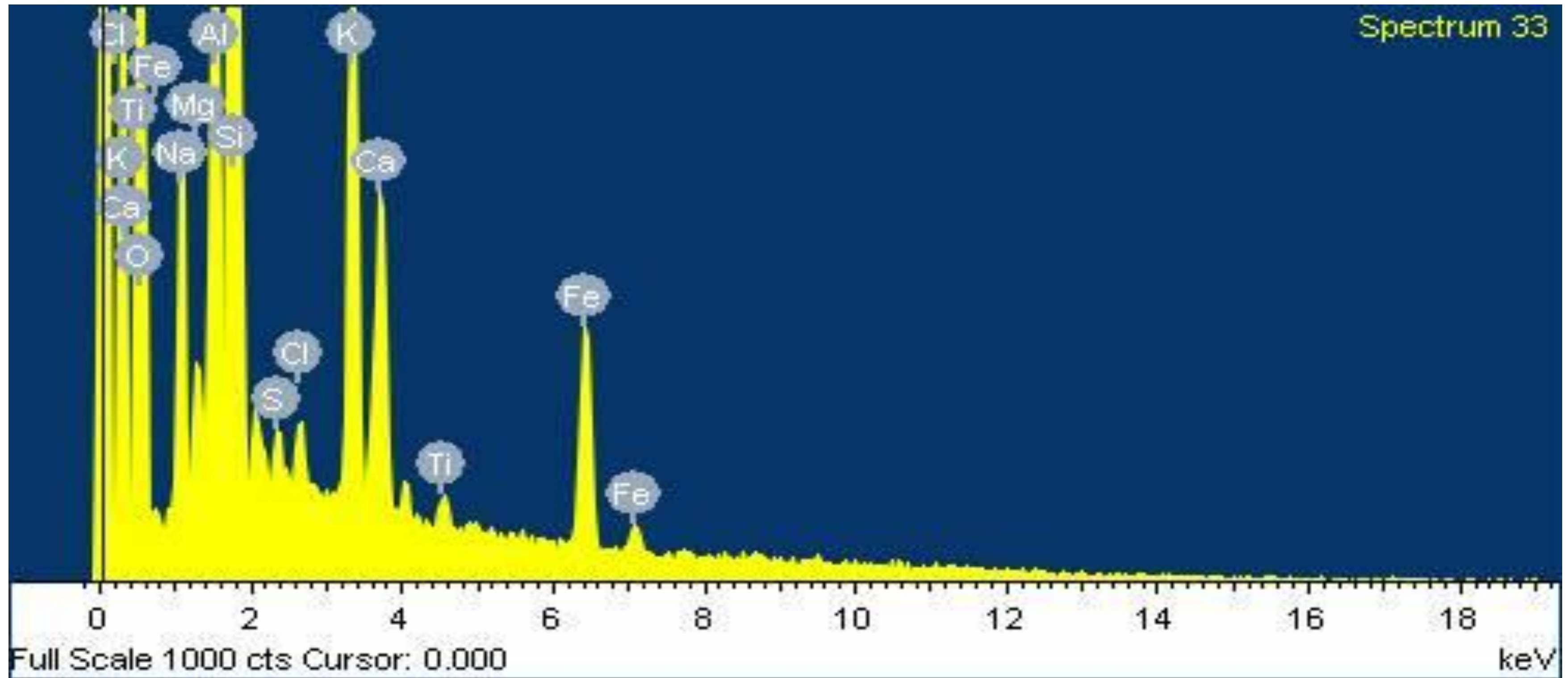


Figure 4.9 SEM-EDS spectra of *M. lyra* guano samples shows the presence of elements such as Cl, K, Ca, Na, S, Fe, Mg, Al and Ti.

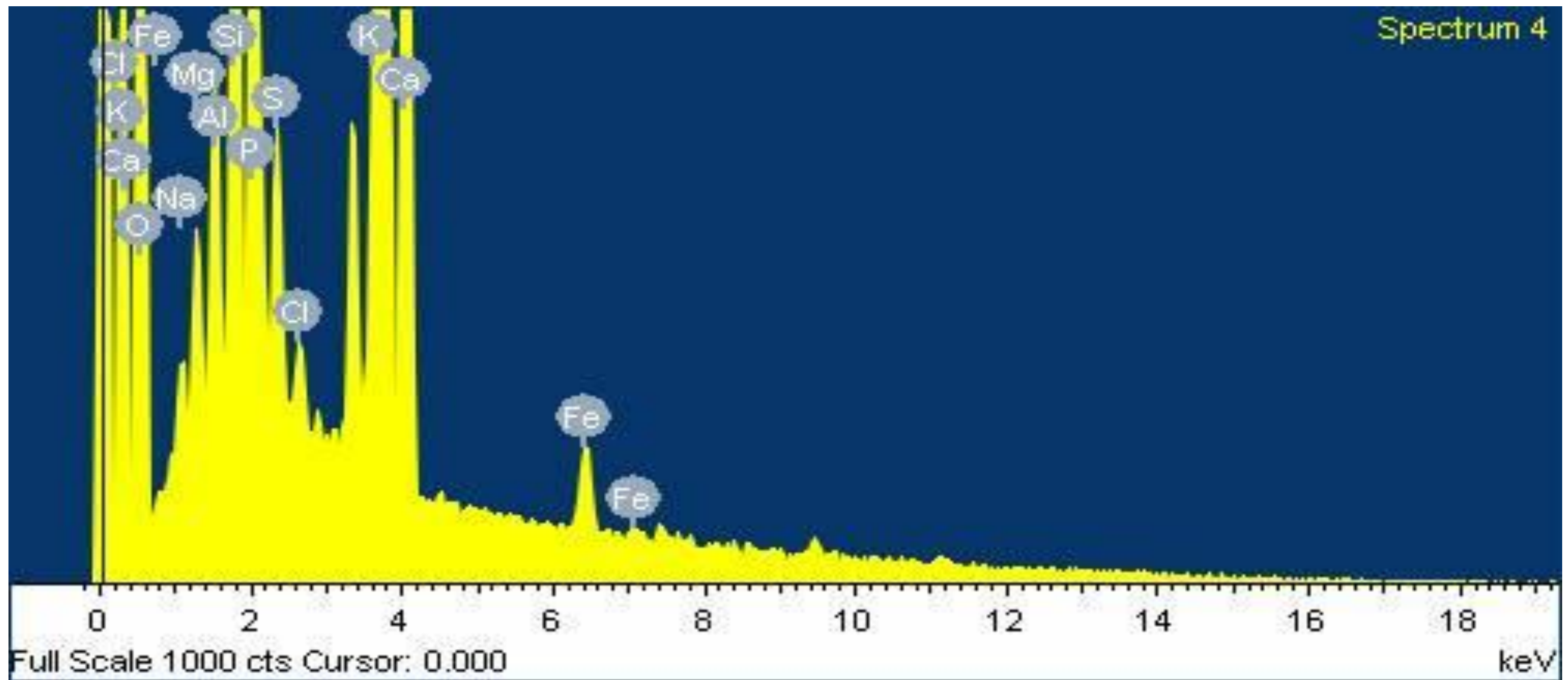


Figure 4.10 SEM-EDS spectra of *H. fulvus* guano samples shows the presence of elements such as Cl, K, Ca, Na, S, Fe, Mg, Al and P.

Summary

Summary

Bats are the second largest order among mammals, divided into two suborder the Megachiroptera (frugivorous) and Microchiroptera (Insectivorous bats). Bats dwell in a wide variety of habitats in both natural and manmade structures. Among mammals, bats play unique role in ecological balance, nutrients cycling and redistribution of forests. The insectivorous bats are called voracious feeders of nocturnal insects including many crop and forest pests. The excreta of wild birds and animals, including bats, contain medically significant fungi. Bat guano is one of the most important substrates for fungi and provides optimal environmental conditions ideal for fungal growth. Fungi play an important role on the decomposing of different substrates, influencing energy flow through the subterranean food.

For the isolation of guanophilic fungi of bats, guano samples were collected from different districts of U.P. The guanophilic fungi were isolated using serial dilution agar plating method and characterize by morphological. For the molecular identification modified CTAB method was followed, using internal transcribed spacer (ITS) gene. Cells were carefully harvested and ground with potassium bromide. Pellets were scanned using Nicolet 670 FT-IR spectrometer. For the diet analysis each pellet was dissolved in distilled water and examined under a binocular light microscope. Photographs of recognizable body parts of prey were taken by light microscope and SEM and identified. For elements analysis guano samples were dried and mounted on metal stub and coated with palladium sputter coater. The samples were analyzed by performing point analysis mode.

A total of 10 bat species such as *R. leschenaulti*, *C. sphinx*, *R. hardwickii*, *R. microphyllum*, *S. heathii, S. kuhlii, P. coromandra*, *M. lyra* and *T. nudiventris* were

observed during the course of study. Among them *C. sphinx*, *R. hardwickii*, *S. heathii, S. kuhlii, P. coromandra* and *M. lyra* were abundant.

A total of 32 species (56 isolates) were isolated from the guano of 10 species of bats. Out of them 18 were isolated from the guano of frugivorous bats while 38 were isolated from the guano of insectivorous bats. In which 12 new strains of guanophilic fungi were reported first time from the guano of bat speciessuch as *A. versicolor* strain PKM25 and PKM16, *A. flavus* strains PKM24, PKY2, PKM15, PKM23, PKM18 and VE1, *A. sydowii* strain PKY1, *P. crustosum* strain PKM19and *A. tenuissima* strain NKG1, besides this first time from the guano of bats 25 new fungusisolates were isolated such as *Aspergillus flavus* isolates VE7, pkm3, pkm11 and pkm2, *A. oryzae* isolate VE9 and pkm8, *A. stellatus* isolate VE6 and Pkm12, *A. sclerotiorum* isolate pkm9, Aspergillus sp. PKM22 and PKM17 and *A. caelatus* isolate pkm10, *P. citrinum* isolate pkm1 and pkm6, *P. oxalicum* isolate VE11 and pkm7, *P. polonicum* isolate Pkm13, *P. capsulatum* isolate pkm14, *P. concentricum* isolate VE4, *P. rubidurum* isolate pkm5, *C. tenuissimum* isolate VE8, *M. indicus* isolate BBAU and *S. implicatum* isolate pkm4.

The results of IR spectroscopy showed the presence of functional groups such as phenol, alcohols, alkene (aromatic) and alkyne in the cell wall of all isolated filamentous fungi. Imines was observed in 19 fungal isolates which belong to genus *Aspergillus*, *Penicellium*, *Cladosporium*, *Chrysosporium*, *Absidia*, *Davidiella*, *Trichocomaceae*, *Periconia*, *Mucor* showed the presence.

Nitrogen was observed in 24 fungal isolates of genus *Aspergillus*, *Penicillium*, *Cladosporium*, *Chrysotropicum*, *Davidiella*, *Trichocomaceae*, *Muccor*, *Malbranchea* and *Trichoderma*. Due to C–C triple bond observed in *Aspergillus* sp. PKM17 and *C.*

tenuissimum isolate VE8 showed the presence of mono-substituted alkyne. Secondary amide (1552.2 cm^{-1} – 1522.1 cm^{-1}) was found in all fungal isolates/strains except the eight isolates isolated from the guano of bats.

Aliphatic groups (1382.8 - 1313.3 cm^{-1}) due to strong bonding of C-NO_2 were found in *Aspergillus*, *Penicellium* and *Muccor*. The results obtained here can serve as a basis for the development of a database for species identification and strain characterization of guanophilic fungi.

Analysis of fecal pellets revealed the presence of insect remenants of order Coleoptera, Hymenoptera, Odonata, Hemiptera, Neuroptera, Lepidoptera and Diptera. The remnants of coleopteran insects were observed in the guano samples of all bat species investigated in this study. Hymenoptera was also recorded in all bat species except *P. coromandra*.Maximum insects order represented in the guano of*S. kuhlii*,out of eight insect orders six insect order were found.

A total of 15 elements such as Aluminum (Al), Boron (Br), Calcium (Ca), Copper (Cu), Chlorine (Cl), Iron (Fe), Potassium (K), Manganese (Mn), Magnesium (Mg), Sodium (Na), Phosphorous (P), Sulpher (S), Titanium (Ti), Zirconium (Zr) and Zinc (Zn) observed in the guano samples of bats. The elements such as Ca, K, Mg, S, P, Cl, Br, Cu, Fe, Mn and Zn found in the guano are essential for the growth of plants. The six macronutrients such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) were abundant in the bat guano. The abundance of macronutrients in bat guano suggests its suitability as bio-fertilizer.

Conclusion

Conclusion

The outcome of this study revealed that the state Uttar Pradesh offer potential roost sites for both insectivorous and frugivorous bats. The occurrence of *C. sphinx*, *R. hardwickii*, *S. heathii*, *S. kuhlii*, *P. coromandra* and *M. lyra* was abundant. Insectivorous bats preferred to roost in tree cavities, wall crevices, roofs of abandoned buildings, historical monuments and caves, while frugivorous bats roosts mostly in buildings in the study area and on few occasions occupied tent roosts. The roost sites of *R. leschenaulti* were stable, undisturbed, and long lasting.

The guanophilic fungi of bats such as *Aspergillus flavus* causes chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis play important role on human health. *Aspergillus versicolor* and *A. niger* were known to cause severe lung problems (Aspergillosis) to human. *Absidia corymbifera* is opportunistic mycoses by causing zygomycosis and most commonly reported as an animal pathogen and causes mycotic abortion in cows. *Cladosporium resinae* actively decomposes hydrocarbons. *Chrysosporium tropicum* is a potent keratinophilic fungus. *Trichoderma* sp. is most promising and an effective biocontrol agent for vegetable diseasesand an antagonist controlling wide range of microbes.

Fourier-transform infrared (FTIR) spectroscopy is a sensitive method for the investigation of fungi biochemical composition. Presence of several –OH group represent the presence phenol and alcoholsbecause cell wall of filamentous fungi is composed of chitin.Polysaccharides are the main constituents of fungal cell walls and some of them, such as β1, 3-D-glucans that also contain some β-1, 6-linked branches and chitin, a linear polymer of N-acetyl-glucosamine, are distributed widely in many species of different genera. The obtained results serve as a basis for the development of

a database for species identification on the basis of chemical composition and strain characterization of fungi.

Insectivorous bats consume a large quantity of insects every night due to high metabolic rate. The intensive foraging of insectivorous bats is well known to reduce the density insects. In this study diet of insectivorous bats leads to the conclusion that various species of bats are selective to certain orders of insects which may lead to the biological control of the target insect pest in agriculture. The insects belong to the orders Coleoptera, Lepidoptera, Homoptera and Hemiptera are the major agricultural pests which were found in the guano of insectivorous bats. The use of biological pest management is more important in current scenario as continuous uncontrolled means of chemical control has led to calamitous consequences.

In many parts of the world, the bat guano is widely used as fertilizer due to the high nitrogen content and bat urine has some nematocidal effect. The elements such as Ca, K, Mg, S, P, Cl, Br, Cu, Fe, Mn and Zn found in the guano are essential for the growth of plants. Out of three essential elements NPK, PK was found in the guano of bats. The six macronutrients such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) were abundant in the bat guano. The abundance of macronutrients in bat guano suggests its suitability as bio-fertilizer. It prompted a novel suggestion to use the guano from selected groups of bats can be used as fertilizer in selective crops to enhance the production and quality.

References

Armstrong D (1993) Treatment of Opportunistic Fungal Infections. Clin Infect Dis 16(1):1–7

Ahearn DG, Meyers SP (1972) The role of fungi in the decomposition of hydrocarbons in the marine environment. In: Walters AH, Vander H, Plas EH (Eds) Biodeterioration of Materials.Applied Science, London

Advani R (1981) Food and Feeding Ecology of the Rat Tailed Bat in the Rajasthan Desert STOSUNKI POKARMOWE U *Rhinopoma microphyllum kinneari*. Acta Theríologica 26(15):269–272

Advani R (1981) Seasonal fluctuations in the feeding ecology of the Indian false vampire *M. lyra* in Rajasthan. Zeitchrift saugetierk 46(2):90–93

Ajello L (1986) Hyalohyphomycosis and phaeohyphomycosis: two global disease entities of public health importance. Eur J Epidemiol 2:243–251

Altingham JD (1996) Bats: biology and behaviour. Oxford University Press; Newyork.

Altringham JD (1996) Bats: biology and behaviour. Oxford University Press

Ashwani KM, Sridhar KR (2002) Towards organic farming with millipede Arthrosphaera magna. Current Science 82:20–22

Adams RA, Scott C, Pedersen, Katherine M, Thibault, Jadin J, Petru B (2003) Calcium as a limiting resource to insectivorous bats: can water holes provide a supplemental mineral source. J Zool 260(2):189–194

Anonymous (2007) Micronutrients soil nutrient management for Maui County.College of Tropical Agriculture and Human Resources.University of Hawai'i.

Al-Abdalall AHA (2009) Production of aflatoxin by *A. flavus* and *A. niger* strains isolated from the seeds of pulses. J Food Agric Environ 7:33–39

Ahmad W, Niaz A, Kanwal S, Rahmatullah MK, Rasheed MK (2009). Role of Boron in Plant Growth: A Review. J Agric Res 47(3)

Amend AS, Seifert KA, Samson R, Bruns TD (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. PNAS, USA 107:13748–13753

Atagazli L, Andrew RG, Wayne M, Aisak GP, Jeffrey MW (2010) Is *Penicillium citrinum* implicated in sago hemolytic disease?. Southeast Asian J Trop Med Public Health 41(3):641–646

Anderson B, Frisvad JC, Sondergaard I, Rasmussen IB, Larsen LS (2011) Associations between fungal species and water-damaged building materials. Appl Environ Microbiol77:4180–4188

Ajay KG, Sharma S, Avasthi S, Bhadauria R (2011) Diversity, Pathogenicity and Toxicology of *A. niger*: An Important Spoilage Fungi. Res J Microbiol 6(3):270–280

Avasn YM, Aruna LK, Ramakrishna RS, Apta CD (2011) *Chrysosporium tropicum* - a potential feather/hair waste degrading keratinophilic fungi. J Environ Res Manag 2:14-18

Brosset A (1962) The bats of central and western India Part 2. Journal of the Bombay Natural History Society 59:583–642

Barbour RW, Davis WH, (1969) Bats of America. University of Kentucky Press, Lexington, Kentucky

Brooke AP (1987) Tent construction and social organization in *Vampyressa nymphaea*.(Chiroptera: Phyllostomidae) in Costa Rica. J Tropical Ecolo 3:171–175

Barclay RMR (1991) Population structure of temperate zone insectivorous bats in relation to foraging behaviour and energy demand. J Anim Ecol 60:165–178

Bhat HR, Kunz TH (1995) Altered flower/fruit clusters of the Kittul palm used as a roost by the short-nosed fruit bat *Cynopterus sphinix* (Chiroptera: Pteropodidae). J Zool (London) 235:597–604

Bates PJJ, Harrison DL (1997) Bats of the Indian Subcontinent. Harrison Zoological Museum Press, England

Brown PH, Shelp BJ (1997). Boron mobility in plants.Plant Soil. 193:85-102

Bunnell JE, Hice CL, Watts DM, Montrueil V, Tesh RB, Vinetz JM (2000).Detection of pathogenic *Leptospira* spp. infections among mammals capturedin Peruvian Amazon basin region.Am J Trop Med Hyg 63:255–258

Bush R, Portnoy JM (2001) The role and abatement of fungal allergens in allergic diseases. J. Allergy Clin Immunol 107:S430–S440

Benda P, Aulagneir S, Palmerini J (2004) *Rhinopoma hardwickii.* Red List Three Spec 2(3):231–256

Bartinicki-Garcia S (2006) Chitosomes past present and future. FEMS Yeast Res 6:957–965

Bowman SM, Free SJ (2006) The structure and synthesis of the fungal cell wall. Bioessays 28:799–808

Balajee SA, Houbraken J, Verweij PE, Hong S-B, Yaghuchi T, Varga J, Samson RA (2007) Aspergillus species identification in the clinical setting. Stud Mycol 59: 39–46

Barclay RMR, Kurta A (2007) Ecology and behavior of bats roosting in tree cavities and under bark. Pp. 17–59 in Bats in forests: conservation and management (MJ Lacki, JP Hayes, and A. Kurta, eds.). Johns Hopkins University Press, Baltimore, Maryland

Baddley JW, Marr KA, Andes DR, Walsh TJ, Kauffman CA, Kontoyiannis DP, Ito JI, Balajee SA, Pappas PG, Bunnell JE, Hice CL, Watts DM, Montrueil V, Tesh RB, Vinetz JM (2009) Detection of pathogenic *Leptospira*spp. infections among mammals capturedin Peruvian Amazon basin region.Am J Trop Med Hyg 63: 255–258

Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, Buckles EL, et al. (2009) Bat white-nose syndrome: an emerging fungal pathogen? Science 323:227

Biswas J, Sharma K, Harris KK, Rajput Y (2013) Biodeterioration agents: Bacterial and fungal diversity dwelling in or on the pre-historic rock-paints of Kabra-pahad, India. Iran J Microbiol 5(3):309–314

Churchill SK (1991) Distribution abundance and roost selection of the orange horseshoe bat, *Rhinonycteris aurantinus*, a tropical cave dweller. Wildl Res 18:343–354

Chandrashekaran MK, Marimuthu G (1994) A huge colomy of the dog faced bats *Rousettus leschenaulti*, South India. Bat Res News 35:485–506

Carbonero ER, Sassaki GL, Stuelp PM, Gorin PAJ, Woranovicz-Barreira SM, Iacomini M (2001) Comparative studies of the polysaccharides isolated from lichenized fungi of the genus Cladonia: significance as chemotypes. FEMS Microbiol Lett 194:65–69

Carson C III, Rittmaster R (2003) The role of dihydrotestosterone in benign prostatic hyperplasia.Urology 61(4:1):2–7

Culver DC, White WB (2005) Encyclopedia of Caves. Elsevier Academic Press, Burlington, California, USA, London, UK. 654

Castro AM, Carvalho ML, Leite SGF, Pereira JN (2010) Cellulases from *Penicillium funiculosum*: production, properties and application to cellulose hydrolysis. J Ind Microbiol Biotechnol 37:151–158

Chen M, Houbraken J, Pan W, Zhang C, Peng P, Wu L, Xu D, Xiao Y, Wang Z, Liao W (2013) Pulmonary fungus ball caused by Penicillium capsulatum in a patient with type 2 diabetes: a case report. BMC Infectious Diseases 13:496

Dyer JR (1965) Applications of Absorption Spectroscopy of Organic Compounds.Prentice - Hall Pub.; Underlined, Notations edition.

Durakovic S, Gallic J, Pajnovic P (1989) Toxic and cancer metabolites of moulds in food and fooder. Hrana I Ishrana 30:71–100

Doern GV, Vautour R, Gaudet M et al. (1994) Clinical impact of rapid in vitro susceptibility testing and bacterial identification. J Clin Microbiol 32:1757–62

Derby RW, Gates DM (1996) The temperature of tree trunks calculated and observed. Am J Bot 53:580–537

Duane AS, Mazin BQ (1996) *Rhinopoma Microphyllum*.Mammalian species.Am Soc Mammal 542:1–5

Davenport RJ, Tester M (2000) A weakly voltage-dependent, nonselective cation channel mediates toxic sodium influx in wheat. Plant Physiol 122:823–83

Demidchik V (2002) Non selective cation channels in plants. Annu Rev Plant Physiol Plant Mol Biol 53:67–107

Entwistle AC, Racey PA, Speakman JR (1997) Roost selection by the brown long-eared bat *Plecotus auritus*. J Appl Ecolo 34:754–763

Engelhart S, Loock A, Skutlarek D, Sagunski H, Lommel A, Farber H, Exner M (2002) Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. Appl Environ Microbiol 68:3886–3890

Easterwood GW (2002) Calcium’s Role in Plant Nutrition. Research shows soluble calcium fertilizer plays vital role in production of high-quality crops. Fluid J 1–3

Elizabeth AHP, Colin FQ, Wiebke T, Mario M, Michela S (2009) Physiological functions of beneficial elements. Curr Opin Plant Biol 12:267–274

Edmondson DA, Barrios CS, Brasel TL, Straus DC, Kurup VP, Fink JN (2009) Immune response among patients exposed to molds.Int J Mol Sci10:5471–5484

Estrada-Bárcenas D, Palacios-Vargas JG, Estrada-Venegas E, Klimov PB, Martínez-Mena A, Taylor ML (2010) Biological activity of the mite Sancassania sp. (Acari:Acaridae) from bat guano associated with the pathogenic fungus *Histoplasma capsulatum*. Memoires do Instituto Oswaldo 105(2)127–131

Elsayed Z, Salem MD, Ibrahim MI, Shahin MD, Yaser F, Mohammed MD, Hamed M, Abdo MD, Mahmoud F, Abdel HMD, Hanaa E, Emam MD, Mohamed F, Abdel SMD (2010) Applicability of Fourier Transform Infrared (FT-IR) Spectroscopy for Rapid Identification of Some Yeasts and Dermatophytes Isolated from Superficial Fungal Infections.J Egypt Women Dermatol Soc 7:2

Fenton M (1983) Roost used by the African insectivorous bat, *Scotophilus leucogaster* (Chiroptera: Vespertilionidae). Biotropica 15:129–132

Flores E, Cabeza M, Quiroz A, Bratoeff E, Garcìac G, Ramìrez E (2003) Effect of a novel steroid (PM-9) on the inhibition of 5α-reductase present in Penicillium crustosum broths. Steroids 68:271–275

Ferrara FJ, Leberg PL (2005) Characteristic of position selection by day-roosting bat under bridges in Louisiana. J Mammol 86:729–735

Fischer G, Braun S, Thissen R, Dott W (2006) FT-IR spectroscopy as a tool for rapid identification and intra-species characterization of airborne filamentous fungi. J Microbiol Methods 64:63–77

Green EE (1907) Bats feeding on birds. J Bombay Nat Hist Soc 18: 190

Gorin PAJ, Spencer JFT (1970) Proton magnetic resonance spectroscopy- an aid in identification and chemotaxonomy of Yeast. Adv Appl Microbial 13:25–89

Gupta UC (1979) Boron nutrition of crops. Adv Agron 31:273–307

Gellman ST, Zielinski WJ (1996) Use by bats of old-growth redwood hollows on the north coast of California. J Mammol 77:255–265

Garcı´a-Hermoso D, Mathoulin-Pelissier S, Couprie B, Ronin O, Dupont B, Dromer F (1997) DNA typing suggestspigeon droppings as a source ofpathogenic *Cryptococcus neoformans* serotype. D J ClinMicrobiol 35:2683–2685

Gunde-Cimerman N, Zalar P, Jeram S (1998) Mycroflora of cave cricket Troglophilus neglectus cadavers. Mycopathologia 141:111–114

Gianni C, Caretta G, Romano C (2003) Skin infection due to *Geomyces pannorum* var. pannorum. Mycoses 46:430–2

Gunn J (2004) Encyclopedia of Cave and Karst Science.2nd ed. Fitzroy Dearborn, Chicago and Lodon, 902p.

Guan H, Yang L, Guo J, Xiaonan Ma, Wang H, You S(2007) Morphological and Molecular Identification of *Aspergillus versicolor* D−1 with Selective Reduction Ability. Asian J Tradit Med 2(1)

Gargas A, Trest MT, Christiensen M, Volk TJ, Blehert DS (2009) *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 108:147–54

Humphrey SR (1975) Nursery roost and community diversity of Nearctic bats. J Mammol 56:321–346

Hagensee ME, Bauwens JE, Kjos B, Bowden RA (1994) Brain abscess following marrow transplantation: experience at the Fred Hutchinson Cancer Research Center, 1984-1992. Clin Infect Dis 19:402–408

Hawksworth DL, Rossman AY (1997) Where are all the undescribed fungi? Phytopathology 87:888–891

Hutchinson JT, Lacki MJ (2001) Possible microclimate benefits of roost site selection in the Red Bat, Lasiurus borealis, in mixed mesophytic forests of Kentucky. Can Field Nat 115(2):205–209

Hutson AM, Mickleburgh SP, Racey PA (2001) Global Status Survey and Conservation Action Plan. Microchiropteran Bats. IUCN, Gland

Hashemi SJ, Emami M (2003) A survey of 800 bats for isolation of *Histoplasma capsulatum* in Iran. Acta Medica Iranica 41:2

Hinman KE, Snow TK (2003) Arizona bat conservation strategies plane. Technical report 213. Nongane and endangered wild life programme. Arizone game and fish department. Phoenix-Arizona

Houbraken JAMP, Frisvad JC, Samson RA (2010) Taxonomy of Penicillium citrinum and related species. Fungal Diversity 44:117–133

Howard HT, Paul RMJR, Veilleux JP, Holt J (2012) Foods of Bats (Family Vespertilionidae) at Five Locations in New Hampshire and Massachusetts. Can Field Nat 126(2):117–124

Johnston IR (1965) The composition of the cell wall of *Aspergillus niger*. Biochem J 96(3):651–658

Jenkins EV, Laine T, Morgon SE, Cole KR, Speakman JR (1998) Roost selection in the pipistrelle bat, *Pipistrellus pipistrellus* (Chiroptera:Vespertilionidae), in northeast Scotland. Anim Behav 56:909–917

King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 4(2):301–307

Kunz TH (1982) Roosting ecology of bats In: Kunz, T.H. (Ed), Ecology of bats. Plenum Press, New York 1–56

Kale RD, Mallesh BC, Bano K, Bagyaraj DJ (1992) Influence of Vermicompost Application on the Available Macronutrients and Selected Microbial Populations in a Paddy Field. Soil Biol Biochem 24:1317–1320

Knudtson WU, Kirkbride CA (1992) Fungi associated with bovine abortion in the northern plains states (USA). J Vet Diagn Invest 4:181–185

Kunza TH, Pierson DP (1994) Bats of the world an introduction, 1-46. In: Nowak, R.W. (ED), Walkers bats of the world. Johns Hopkins University Press, Baltimore Maryland USA, 287

Keleher H, Sara A (1996) Guano: bats' gift to gardeners. Bats 14:15–17

Kurup VP, Shen HD, Banerjee B (2000) Respiratory fungal allergy. Microbes Infect 2:1101–1110

Kerth G, Weissmann K, König B (2001) Day roost selection in female Bechstein's bats (Myotis bechsteinii): a field experiment to determine the influence of roost temperature. 126(1):1–9

Kunz TH, Lumsden LF (2003) Ecology of cavity and foliage roosting bats.InT. H. Kunz and MB Fenton (Eds.).Bat ecology. University of Chicago Press, Chicago 3–87

Kurup VP (2003) Fungal allergens. Curr Allergy Asthma Rep. 3:416–423

Kis-Papo T, Kirzhner V, Wasser SP, Nevo E (2003) Evolution of genomic diversity and sex at extreme environments: fungal life under hyper saline Dead Sea stress. PNAS, USA. 100:14970–14975

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the Fungi. 10th Edition. CAB International, Wallingford, UK

Lim TK, Rohrbach KG (1980) Role of *Penicillium funiculosum* strains in the development of pineapple fruit diseases. Phytopath 70:663–665

Loomis WD, Durst RW (1992) Chemistry and biology of boron. Bio Factors 4:229–239

Larone DH (1995) Medically important fungi. ASM Press. Washington

Lee Y, Ling-Ling LYL, Lee L (2005) Food habits of Japanese pipistrelles *Pipistrellus abramus* (Chiroptera: Vespertilionidae) in northern Taiwan. Zool Stud 44(1): 95–101

Lee Y, Lee L (2005) Food Habits of Japanese Pipistrelles Pipistrellus abramus (Chiroptera: Vespertilionidae) in Northern Taiwan. Zool Stud 44(1): 95–101

Levin E, Yom-Tov Y, Barnea A (2008) Frequent summer nuptial flights of ants provide a primary food source for bats. Naturwissenschaften.

Ludmilla MSA, Yasmine A (2008) Diet of two sympatric insectivores bats (Chiroptera: Vespertilionidae) in the Cerrado of Central Brazil. Revista Brasileira de Zoologia 25 (1): 28–31

Leonardo DM, Alves VS, Costa FLB (2012) Molecular identification of filamentous fungi in guano of haemotofagous and insectivores bats in Lapinha´s cave – MG. ISIMPÓSIO BRASILEIRO DE IDENTIFICAÇÃO MOLECULAR DE ESPÉCIES. 10 e 11 de Rafain Palace Hotel & Convention Center - Foz do Iguaçu – PR

Lecellier A, Mounier J, Gaydoua V, Castrec L, Barbier G, Ablainc W, Manfait M, Toubas, Sockalingum GD (2014) Differentiation and identification of filamentous fungi by high-throughput FTIR spectroscopic analysis of mycelia. Int J Food Microbiol 168(169):32–41

Mccann C (1934) The Indian vampire (Megaderma lyra) feeding on a Pipistrelle. J Bombay Nat Hist Soc 37:479

Michell AJ, Sourfield G (1970) An assessment of infrared spectra as indicators of fungal cell wall composition. Aust J bioI Sci 23:345–60

Martínez DR (1972) Histoplasmosis aguda—Epidemia en San Germán. Boletín de la Asociación Médica de Puerto Rico, 64:291–294

Martin B (1976) The influence of patterns of guano renewal on bat guano arthropod communities. Cave Research Foundation Annual Report 36–42

Mok WY, Regina C, Luizao C, Silva MSB (1982) Isolation of Fungi from Bats of the Amazon Basin. Appl Environ Microbiol 44(3):570–575

Moller EM, Bahnweg G, Sandermann H, Geiger HH (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies and infected plant tissues. Nucleic Acids Res 20(22):6115–6116

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. London: Academic Press

Marschner H (1995) Mineral Nutrition of Higher Plants.Aca. Press, San Diego, USA, 379–396

Kümmerle M, Scherer S, Seiler H (1998) Rapid and Reliable Identification of Food-Borne Yeasts by Fourier-Transform Infrared Spectroscopy. Appl Environ Microbiol 64(6):2207–2214

Mattsson R, Haemig PD, Olsen B (1999) Feral pigeons as carriers of *Cryptococcuslaurentii*, *Cryptococcus uniguttulatus*and *Debaryomyces hansenii*. MedMycol 37:367–369

Margarita P, Quinteiro R (2000) “Fourier Transform Infrared (FT-IR) Technology for the Identification of Organisms”, Clin Microbiol Newsletter 22:8

Maquelin K, Kirschner C, Choo-Smith LP, et al. (2003) Prospective study of the performance of vibrational spectroscopies for rapid identification of bacterial and fungal pathogens recovered from blood cultures. J Clin Microbiol 41(1):324–329

Martin KJ, Rygiewicz PT (2005) Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiol 5:28

Essendoubia M, Toubasb D, Bouzaggoua M, Pinonb J, Manfaita M, Sockalinguma GD (2005)Rapid identification of Candida species by FT-IR microspectroscopy.Biochimica et Biophysica Acta 1724:239–247

Mbata TI (2008) Isolation of fungi in hyper saline Dead Sea water.Sudanese J Publ Health 3:170–172

Movasaghi Z, Rehman S, Rehman IU (2008) Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues. Appl Spect Rev 43:134–179

Matsumoto T, Ajello L, Matsuda T, Szaniszlo PJ, Walsh TJ (1994) Developments in hyalohyphomycosis and phaeohyphomycosis. J Med Vet Mycol 32:329–349

Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green DE, Shearn- Bochsler V, et al. (2009) Histopathologic criteria to confirm white-nose syndrome in bats. J Vet Diagn Invest 21:411–4

Moreno G, Arenas R (2010) Other fungi causing Onychomycosis. Clin Dermatol28:160–163

Nicolai V (1986) The bark of tree: thermal properties, microclimate and fauna. Oecologia 69:148–160

Northup DE, Lavoie K, Mallory L (1997) Microbes in caves. Nat Speleol Soc News 55:11

Norberg UM (1990) Vertebrate flight. Springer – verlag, Berlin

Naumann D, Helm D, Labischinski H (1991) Microbiological characterizations by FT-IR spectroscopy.Nature351:81–2

Nobel DJG, Van den Ende H, Klis FM (2000) Cell wall maintenance in fungi. Trends Microbiol 8:344–345

Naumann A, Navarro-González M, Peddireddi S, Kües U, Polle P (2005) Fourier transform infrared microscopy and imaging: Detection of fungi in wood. Fungal Genet Biol 42:829–835

Noonimabe P, Mahakarnchanakulb W, Niesend KF, Frisvadd JC, Samsona RA (2009) Fumonisin B2 production by *Aspergillus niger* in Thai coffee. Food Addit Contam 26:94–100

Ohnishi J-I, Flugge U-I, Heldt HW, Kanai R (1990) Involvement of Na^+ in active uptake of pyruvate in mesophyll chloroplasts of some C4 plants. Plant Physiol 94:950–959

Oliveira CM (2005) Aspectos bioecológicos do coró-das-hortaliças *Aegopsis bolboceridus* (Thomson) (Coleoptera: Melolonthidae) no Cerrado do Brasil Central. Planaltina, Embrapa Cerrados, Série Documentos 143:28

Phillips WWA (1922) Notes on the habits of some Ceylon bats. J Bombay Nat Hist Soc 28: 448–452

Prakash I (1959) Foods of the Indian false vampire. J Mammal 40:545–547

Prakash I (1961) Taxonomic and biological observations on the bats of the Rajasthan Desert. Records of Indian Museum 59:149–170

Poulson TL (1972) Bat guano ecosystems. Bulletin of the National Speleological Society, 72:55–59

Phillips W (1980) Manuals of the mammals of Sri Lanka.Part I. Wildlife and nature protection society of Sri Lanka 1 – 116. Phylogenetic relation between microbats, megabats and primates (Mammalia: Chiroptera and primates). Philosphical Transaction R Soc 325:489–559

Paice MG, Jurasek L (1984) Removing hemicellulose from pulps by specific enzymic hydrolysis. J Wood Chem Technol 4:187–198

Pavey CR, Burwell CJ, Grunwald J et al (2001) Dietary benefits of twilight foraging by the insectivorous bat *Hipposideros speoris*. Biotropica 33:670–681

Parsons S, Lewis KJ, Psyllakis JM (2003) Relationships between roosting habitat of bats and decay of aspen in sub-boreal forests of British Columbia. Forest Ecol Manage 177:559–570

Pfaller MA, Diekema DJ (2004) Rare and Emerging Fungal Pathogens: Concern for Resistance beyond *Candida albicans* and *Aspergillus fumigatus*. J Clin Microbiol 42(10):4419-4431

Perri P, Campa C, Incorvaia C, Parmeggiani F, Lamberti G, Costagliola C, Sebastian A (2005) Endogenous *Aspergillus versicolor* endophthalmitis in an immuno-competent HIV-positive patient. Mycopathol 160:259–261

Paul SM, Sagniko F (2008)The use of bat guano in the improvement of the nutritive value ofpoor quality roughage fed to ruminants in Tanzania. Veterinarski Arhiv78(5):417–427

Pitt JI, Hocking AD (2009) Fungi and food spoilage. Springer, Berlin, Germany

Paul R, Moosman JR, Thomas HW, Veilleux JP (2012) Diet of the widespread insectivorous bats *Eptesicus fuscus* and *Myotis lucifugus* relative to climate and richness of bat communities. J Mammal 93(2):312–328

Paul RH, Bohmann K, Thomas M, Gilbert P, Zepeda-Mendoza ML, Razgour O, Jones G (2014) Second generation sequencing and morphological faecal analysis reveal unexpected foraging behaviour by Myotis nattereri (Chiroptera, Vespertilionidae) in winter. Front Zool 11:39

Ponmalar S, Vanitharani J (2014) Insect pest management by the horse shoe bats (Rhinolophus species) in the forest ecosystem of Kalakad Mundanthurai Tiger Reserve, India.SIRJ-BES 1(5):2348–5787

Reisenauer HM, Walsh LM, Hoeft RG (1973) Testing soils for Sulfur, Molybdenum and Chlorine. In Soil Testing and Plant Analysis, ed. LM Walsh, JD Beaton, Adison, Wis: Soil Sci Soc Amer173–200

Richard JE(1977) Fundamental of entomology (4th ed.) Prentice-Hall, Upper Saddle River, New Jersey, **10**:0130480304

Racey PA, Speakman JR, Swift SM (1987) Reproduction adaptation of hetrothermic bats at northern boarders of their distribution. S Afr L Sci 83:635– 638

Ransome RD (1990) The natural history of hibernating bats. London: Christopher Helm

Raper KB, Thom C, Fennel DI (1949) A Manual of Penicillia. Baltimore Press Inc. USA 63

Rao M, Gaikwad S, Mishra C, Deshpande V, (1988) Induction and catabolite repression of cellulase in *Penicillium funiculosum*. Appl Biochem Biotech 19:129–137

Ribes JA, Vanover-Sams CL, Baker DJ (2000) Zygomycetes in human disease. Clin Microbiol Rev13:236–301

Rick AA, Pedersen SC, Thibault KM, Jadin J, Petru B (2003) Calcium as a limiting resource in insectivorous bats: can water holes provide a supplemental mineral source? J Zool Lond 260:189–194

Rozynek P, Gilges S, Brüning T, Wilhelm M (2004) Quality test of the Micro SEQD2LSU fungal sequencing kit for the identification of fungi. Int J Hyg Environ Health 207(3):297–299

Ramanujam ME, Verzhutskii B (2004) The prey of the Greater false vampire bat *M. lyra* E. Geoffroy at Kaliveli,Tamilnadu. Zoos' Print J 19(10):1655–1656

Rai V, Mehrotra S (2005) Toxic contaminants in herbal drugs. Environ News Arch 11:1–3

Resende-Stoianoff MA, da Silva DL, Taylor ELS, Reis MF, Magalhaes TFF, Martins CVB, da Silva CM, Santos DA, de Fátima A (2012) Activity of aldimines against isolated from bat guano in a neotropical touristiccave. Mycoses 55:(S1)Ç102–103

Roberto NM, Carolina AB, Lídia MMSA, Nei P (2013) Cellulase production by *Penicillium funiculosum* and its application in the hydrolysis of sugar cane bagasse for second generation ethanol production by fed batch operation. J Biotech 163:38–44

Rahman FU, Perveen F, Rauf T, Salim M, Ali Z, Khan S, Yar MS, Khan NI (2015) Habitat preferences and roost site selection of *Rhinopoma hardwickii* Gray, 1831 in Northwestern, Pakistan. J Anim Plant Sci 25(3–2): 429–432

Sluiter JW, Voute AM, Heerdt PFV (1973) Hibernation of *Nyctalus noctula*. Periodicum Biologorum 75: 71–79

Samson RA (1974) *Paecilomyces* and some allied hyphomycetes. Stud Mycol 6:1–119

Sinha NK (1981) Checklist of Mammals of India. Zoological Survey of India, Kolkata 289

Swift SM, Racey PA, Avery MI (1985) Feeding ecology of *Pipistrelluspipistrellus* (Chiroptera: vespertilionidae) during pregnancy and lactation. Ii. Diet. J Anim Ecol 54:217–225

Sockalingum GD, Bouhedja W, Pina P, Allouch P, Mandray C, Labia R, Millot JM, Manfait M (1997) ATR-FTIR spectroscopic investigation of imipenem-susceptible and -resistant *Pseudomonas aeruginosa* isogenic strains. Biochem Biophys Res Commun 232:240–246

Studier EH, Steven HS, Deanne MR, Don EW (1994) Mineral and nitrogen concentration in feces of some neotropical bats. Journal of Mammal 75: 674–680

Schmalreck AF, Trankle P, Vanca E, Blaschke-Hellmessen R (1998) Differentiation and characterization of Yeasts pathogenic for humans (*Candida albicans*, *Exophiala dermatitidis*) and algae pathogenic for animals (Prototheca spp.) using Fourier transform infrared spectroscopy (FTIR) in comparison with conventional methods. Mycoses 41 (1):71–77

Simmons J (1998) Balancing soil nutrition. Turf Net Monthly. EarthWorks News and Events

Sedgeley JA, O'Donnell CFJ (1999) Roost selection by long tailed bat, *Chalinolobous tubercualtus*, in temperate New Zealand rainforest and implications for the conservation of bats in managed forest. Biol Conserv 88:261–276

Stewart NJ, Munday BL, Hawkesford T (1999) Isolation of *Mucor circinelloides* from a case of ulcerative mycosis of platypus (*Ornithorhynchus anatinus*), and a comparison of the response of *Mucor circinelloides* and *Mucor amphibiorum* to different culture temperatures. Med Mycol 37:201–206

Shen H, Lin WL, Tam MF, Wang SR, Tzean SS, Huang MH, Han SH (1999) Characterization of allergens from *Penicillium oxalicum* and *P. notatum* by immune blotting and N-terminal amino acid sequence analysis. Clin Exp Allergy 29:642–651

Santos A, Marquina D, Leal JA, Peinado JM (2000) 1-6 β-d-glucan as cell wall receptor form *Pichia membranifaciens* killer toxin. Appl Environ Microbiol 66:1809–1813

Sedgeley JA (2001) Quality of cavity microclimate as a factor influencing selection of maternity roosts by a tree-dwelling bat, Chalinolobus tuberculatus, in New Zealand. J Appl Ecol 38:425–438

Shüβler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Schuster E, Dunn-Coleman N, Frisvad J, Dijck P Van (2002) On the safety of *Aspergillus niger*. A review. Appl Microbiol Biotech 59:426–435

Shelton BG, Kirkland KH, Dana W (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States.Appl Environ Microbiol68:1743–1753

Speakman JR, Thomas DW (2003) Physiological ecology and energetics of bats. In: Kunz, T. H., editor. Ecology of bats. New York, NY: Plenum Press 430

Sandt C, Sockalingum GD, Aubert D, Lepan H, Lepouse C, Jaussaud M, Leon A, Pinon JM, Manfait M, Toubas D (2003) Use of Fourier-Transform Infrared Spectroscopy for Typing of *Candida albicans* strains isolated in Intensive Care Units. J Clin Microbio 954–959

Saad DS, Kinsey GC, Kim S, Gaylarde CC (2004) Extraction of genomic DNA from filamentous fungi in biofilms on water-based paint coatings. International Biodeterioration Biodegrad 54:99–103

Salle V, Lecuyer E, Chouaki TE et al (2005) *Paecilomyces variotii*fungemia in a patient with multiple myeloma: case report and literaturereview. J Infect 51:e93–e95

Sridhar KR, Ashwini KM, Seen S, Sreepada KS (2006) Manure quality of guano of insectivorous cave bat *Hipposideros speoris*. Trop Subtrop Agroecosystems 6: 103–110

Sophia E (2010) Foraging behaviour of the microchiropteran bat, *Hipposiderosteron* chosen insect pests. J Biopesticides 3(1):068–073

Srinivasulu B, Srinivasulu C, Venkateshwarlu P (2010) First record of Lesser Yellow House Bat *Scotophilus kuhlii* Leach, 1821 from Secunderabad, Andhra Pradesh, India with a note on its diet. J Threatened Taxa 2(10):1234–1236

Sajid MA, Khan IA, Rauf U (2006) *Aspergillus fumigatus* in commercial poultry flocks, a serious threat to poultry industry in Pakistan. J Anim Pl Sci 16:3–4

Serdar B, Bilen M, Bardhan S (2011) The effects of boron management on soil microbial population and enzyme activities. Afr J Biotech 10(27):5311–5319

Shetty S, Sreepada KS (2013) Prey and Nutritional Analysis of *Megaderma lyra*Guano from the West Cast of Karnataka, India.Adv Biores l4(3):01–07

Thom C, Raper KB (1945) A manual of Aspergilla. Baltimore Press Inc. USA 45

Tuttle MD, Stevenson. 1982. Growth and survival of bats. In: Ecology of bats (Ed. By T. H. Kunz), Plenum Press, NY 105–150

Tidemann CR, Flavel SC (1987) Factors Affecting Choice of Diurnal Roost Site by Tree-Hole Bats (Microchiroptera) in Southeastern Australia. Aust Wildl Res 14(4):459–473

Thompson J, Higgins D, Gibson T (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-speci®c gap penalties and weight matrix choice. Nucl Acids Res 22:4673–4690

Timmins EM, Howell SA, Alsberg BK, Noble WC, Goodacre R (1998) Rapid differentiation of closely related *Candida* species and strains by pyrolysis-mass spectrometry and Fourier transform-infrared spectroscopy. J Clin Microbiol 36:367–74

Trabulsi LR, Alterthum F (2004) Atheneu, São Paulo, SP (4thed). Microbiologia 718

Truckesses MW, Scoot PM (2009) Mycotoxins in botanical and dried fruits: A review. Addit Cont 25:181–192

Taylor ELS, Resende-Stoianoff MA, Ferreira RL (2013) Mycological study for a management plan of a neotropical show cave (Brazil). Int J Speleol 42(3):267–277

Usman K (1998) Roosting height and temperature in the roosting ecology of tropical microchirepteran bats. Proceeding Indian Academy of Sciences, Animal Sciences 97:551–560

Vaughan TA (1987) Behavioural thermoregulation in the Africa yellow-winged bat. J. of Mammal 68:376–378

Vonhof MJ, Barclay RMR (1996) Roost-site selection and roosting ecology of forest-dwelling bats in southern British Columbia. Can J Zool 74:1797–1805

Van der Sluis TM, Meuleman EJH, Van Moorselaar RJA, Hong NB, Blankenstein MA, Heijboer AC, Andr éN V (2011) Intraprostatic testosterone and dihydrotestosterone. Part II: concentrations after androgen hormonal manipulation in men with benign prostatic hyperplasia and prostate cancer. BJU International 109:183–188

Vanderwolf KJ, Malloch D, McAlpine DF, Forbes J (2013) A world review of fungi, Yeasts, and slime mold in caves. Int J Speleol 2(1):77–96

Visagie CM, Houbraken J, Frisvad JC, Hong SB, Klaassen CHW, Perrone G, Seifert KA , Varga J, Yaguchi T, Samson RA (2014) Identification and nomenclature of the genus *Penicillium*. Stud Mycol 78:343–371

Weinding R (1934) Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopath 24:1153–1179

Whitaker JO (1978) Food habit analysis of insectivorous bats In: Ecological and behavioural methods for the study of bats, (Kunz, T. H. ed.), Smithsonian Press, Washington. 171–189

Whitaker JO Jr (1995) Food of the big brown bat, *Eptesicus fuscus*, from maternity colonies in Indiana and Illinois. Am Midland Nat134:346–360

Williams LM, Brittingham MC (1997) Selection of maternity roost by big brown bat. J Wildl Manag 61:359–368

Whitaker JO Jr. (2004) Prey selection in a temperate zone insectivorous bat community. J Mamm 85:460–469

Xu G, Magen H, Tarchitzky J, Kafkafi V (2000) Advances in chloride nutrition. Adv Agro 68:96–150

Yamamoto Y, Kohno S, Koga H et al (1995) Random amplified polymorphic DNA analysis of clinically and environmentally isolated *Cryptococcus neoformans* in Nagasaki. J Clin Microbiol 33:3328–3332

Yapa WB, Ratnasooriya WD, Goonatilake MRMPN (2005) Evidence of fish in the diet of Greater false vampire bat *M. lyra* in Sri Lanka. Zoos' Print J 20(4):1834–1835

Yoder JA, Benoit JB, Christiensen BS, Croxall TJ, Hobbs HH (2009) Entomopathogenic fungi carried by the cave or bweaver spider, Meta ovalis (Araneae, Tetragnathidae), with implications formycoflora transer to cave crickets. J Cave Karst Stud 71(2):116–120

Publications

1. Pawan Kumar Misra, Vadamalai Elangovan (2015). **Guanophilic fungi of mouse-tailed bats.** S. Asian J. Life Sci. 3(2): 56-62.

2. Pawan Kumar Misra, Aradhna Singh and Vadamalai Elangovan (2016). **Diversity of Guanophilic Fungi of the Greater Asiatic Yellow Bat, *Scotophilus heathii.*** *Trends in Biosciences 9(1): 09-13.*

3. Pawan Kumar Misra and Vadamalai Elangovan (2016**). Light and Scanning Electron Microscopic Studies on Food Habit Analysis of Insectivorous Bats**. Advances in Life Sciences, 5(9):

4. Pawan Kumar Misra, Divya Sharma and Vadamalai Elangovan (2016**). Diversity of guanophilic fungi of frugivorous bats. In Press.**