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

SUMMARY

of

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

LUCKNOW

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

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 dwell in a wide variety of habitats in both natural and manmade structures and spend more than half of their lives in roost. Many natural and anthropogenic factors influence the roost selection. 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. In many parts of the world, bat guano is widely used as fertilizer due to the high nitrogen content and bat urine has some nematocidal effect.

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. Guanophilic fungi of bats usually serve as saprotrophs or pathogens or as transient chemo heterotrophic microorganisms. Fungi play an important role on decomposing different substrates, influencing energy flow through the subterranean food.

Morphological identification is the 1st step in fungal identification because without knowing the genus fungus specific media cannot be used. On the behalf of specific media particular type of color, spores and texture appear that's the key feature for further identification. Due to the culture limitations, misidentifications in culture collections, and unexplored habitats, only about 5% of fungal species have been accurately described. Molecular methods based on DNA analysis can reveal fungal diversity in ecosystems, and offer the potential benefits of highly sensitive and rapid detection.

The essential feature of the FTIR analysis was to determine the spectra which would permit to differentiate the various species based on their spectra. FTIR spectroscopy a promising clinical approach, because this is conventional, time and money saving, has great identification and discriminating potentials.

There were very little information 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.

Guano samples of bats were collected from different districts of U.P. culture of guanophilic fungus was carried out by serial dilution agar method.The species recognition was carried out by morphological, molecular and spectroscopy methods. DNA was extracted from fungal mycelia using modified CTAB (Moller *et al*., 2010). DNA was amplified and sequenced using Sanger dioxi method. Obtained sequences of these isolates were compared with reference fungal taxa at NCBI database. Phylogenetic trees were constructed using maximum parsimony and Bayesian methods based on internal transcribed spacer (ITS) sequences. Cells of fungal isolates were ground with potassium bromide the pellets were scanned using Nicolet 670 FT-IR spectrometer. The obtained peak frequency was assessed by following Dyer (1965).

The pellets analysis was performed by dissolving individual pellets in distilled water and isolating the recognizable body parts of insects. The body parts of insects photographed using Light microscope and Scanning Electron Microscope and identified up to order level. For elemental analysis, the aseptically collected guano

samples were dried and mounted on metal stub and coated with palladium sputter coater. The samples were analyzed by performing point analysis mode.

In the present study a total of 10 bat species such as *Rousettus leschenaulti*, *Cynopterus sphinx*, *Rhinopoma hardwickii*, *R. microphyllum*, *Scotophilus heathii*, *S. kuhlii*, *Pipistrellus coromandra*, *Megaderma lyra* and *Taphozous nudiventris* were observed. Bats were using historical monuments, old temples, abandoned buildings, caves, crevices, tree holes and foliage roosts as their roost. 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. 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. The current study also revealed that roost sites of *R. leschenaulti* were stable, undisturbed, and long lasting. The average relative temperature and humidity of the roost sites of frugivorous bats were 30.50 ± 1.91 °C and 65.50 ± 7.01 %, while insectivorous bats 30.50 ± 2.68 °C and 72.17 ± 7.01% were recorded respectively.

A total of 56 isolates of fungus belong to 32 species, 13 genus and 9 family isolated from the guano of 10 species of bats. Out of them 18 isolates were isolated from the guano of frugivorous bats while 38 isolates were isolated from the guano of

insectivorous bats. In which 12 new strains such as PKM25 and PKM16 of *A. versicolor*, PKM24, PKY2, PKM15, PKM23, PKM18 and VE1 of *A. flavus*, *A. sydowii* PKY1, *P. crustosum* PKM19 and *A. tenuissima* NKG1 of guanophilic fungi were reported first time from the guano of bat.

Besides 25 new fungal isolates of *Aspergillus flavus* VE7, pkm3, pkm11 and pkm2, *A. oryzae* VE9 and pkm8, *A. stellatus* VE6 and Pkm12, *A. sclerotiorum* pkm9, *A. caelatus* pkm10, *Aspergillus* sp. PKM22 and PKM17, *P. citrinum* pkm1 and pkm6, *P. oxalicum* VE11 and pkm7, *P. polonicum* Pkm13, *P. capsulatum* pkm14, *P. concentricum* VE4, *P. rubidurum* pkm5, *C. tenuissimum* VE8, *M. indicus* BBAU and *S. implicatum* pkm4 were isolated 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 immune compromised patients. *Aspergillus versicolor* and *A. niger* were known to cause severe lung problems (Aspergillosis) to human. *A. niger* causes several ailments of liver, kidney, nervous system, muscles, skin, respiratory organs, digestive tract and genital organs in human. *Absidia corymbifera* is opportunistic mycoses causes 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 diseases and an antagonist controlling wide range of microbes. Thus, the results of current study reveal the diversity of guanophilic fungi of bats and their role on ecosystem and human health.

The IR absorption spectra found from wave number 3440.1 cm^{-1} to 3377.2 cm^{-1}(due to bonded O–H stretching) and 1415.7 - 1400.1 cm^{-1}(weak C–O/ O–H bending) were found in the cell wall of filamentous fungi showed the presence of phenol and alcohols.

N–H stretching between 3395.2 cm^{-1} and 3297.8 cm^{-1} was observed in 19 fungal isolates belong to genus *Aspergillus, Penicellium, Cladosporium, Chrysosporium, Absidia, Davidiella, Trichocomaceae, Periconia* and *Mucor* showed the presence of imines.

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 of genus *Aspergillus, Penicillium, Cladosporium, Chrysotropicum, Davidiella, Trichocomaceae, Muccor, Malbranchea* and *Trichoderma* 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.

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 showed the presence of alkyne.

The absorption bands from 1552.2 cm^{-1} – 1522.1 cm^{-1} in all fungal isolates/strains except the eight isolates showed the presence of secondary amide.

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.

The IR absorption 1382.8 - 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* isolateVE6, *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 (nitro compounds) groups.

The absorption bands of 1315.3 cm^{-1} was observed in only *A. oryzae* isolatepkm8 showed methyl C-H stretching and presence of amide II.

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 from the mentioned bat guano revealed that Coleoptera, Hymenoptera, Odonata, Hemiptera, Neuroptera, Lepidoptera and Diptera insect's orders were present. Insects belong to Coleoptera, Hymenoptera and Odonata were pre-dominant. 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 insect order represented in the guano of *S. kuhlii*, out of eight insect orders six insect orders were found. The 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 insects. Thus, the study supports conserve the bats in their natural habitat.

A total of 15 elements such as Aluminum (Al), 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. 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.

Research Article

Guanophilic Fungi of Mouse-Tailed Bats

Pawan Kumar Misra, Vadamalai Elangovan*

Department of Applied Animal Sciences, School for Bioscience and Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rai Bareli Road, Lucknow, Uttar Pradesh -226025, India.

Abstract | Bats utilize a wide range of structures as their roosting sites and occupy various food niches. The guanophilic fungi of insectivorous bats such as *Rhinopoma hardwickii* and *R. microphyllum* were isolated and identified morphologically using phase contrast and scanning electron microscopes. The fungal culture was carried out using potato dextrose agar (PDA) and species such as *Aspergillus versicolar gr.*, *A. flavus, A. niger*, *Aspergillus* sp., *Penicillium funiculosum, Penicillium* sp., *Absidia corymbifera, Cladosporium cladosporiodes, C. resinae, Chrysosporium tropicum*, *Paecilomyces varitii*, *Malbranchea* sp., *Trichoderma* sp., *Mucor* sp. and yeast were isolated and characterized. *Chrysosporium tropicum* plays a vital role in degrading the insectivorous bat guano which has rich contents of keratin, while *C. resinae* decomposes the hydrocarbons available in the guano. Many other fungi isolated in this study are opportunistic and some are medically and environmentally important.

Keywords | Fungi, Guano, Insectivorous bats, Rhinopomatidae, Scanning electron microscope

Editor | Muhammad Nauman Zahid, Quality Operations Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan.
Received | September 04, 2015; **Accepted** | December 28, 2015; **Published** | December 30, 2015
***Correspondence** | Vadamalai Elangovan, Department of Applied Animal Sciences, School for Bioscience and Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rai Bareli Road, Lucknow, Uttar Pradesh, India; **Email:** elango70@yahoo.com
Citation | Misra PK, Elangovan V (2015). Guanophilic fungi of mouse-tailed bats. S. Asian J. Life Sci. 3(2): 56-62.
DOI | http://dx.doi.org/10.14737/journal.sajls/2015/3.2.56.62
ISSN | 2311–0589

INTRODUCTION

Bats of family *Rhinopomatidae* are known as mouse tailed bats. They are composed of a single genus (*Rhinopoma*) with a large geographical range covering a part of tropical Africa and most of the southern Mediterranean, Middle East and southern Asia (Hill, 1977). The family *Rhinopomatidae* consists of three species of bats, namely *Rhinopoma hardwickii* (lesser mouse-tailed bat), *R. microphyllum* (greater mouse-tailed bat) and *R. muscatellum* (small mouse-tailed bat). They are relatively small and live in arid and semiarid habitats, where they roost in large numbers in caves and man-made structures including houses, wells, tunnels and tombs. They feed on insects, such as flies and beetles. Having such an ecologically significant position, they play an important role as pest-controller.

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. 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 (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). 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 rhinopomatid bats and their pathogenic and ecological role in the ecosystem.

MATERIALS AND METHODS

The guano samples of *R. hardwickii* were collected from Jhushi fort and Khusurubagh fort at Allahabad (25.45°N, 81.85°E), Atala mosque at Jaunpur (25.73°N, 82.68°E), Diyara fort at Sultanpur (26.45°N, 82.11°E) and Thar Ganga Ghat at Varanasi (25.28°N, 82.95°E), while the guano samples of *R. microphyllum* were collected from a natural cave at Chitrakoot (25.00°N, 80.83°E). The population size of *R. hardwickii* colonies ranged from 600 -

Table 1: Colony characteristics of guanophilic fungi isolated from the guano of rhinopomatid bats

Fungal species	Colony diameter (mm)	Colony character		Zonation	Sporulation
		Surface colour	Texture		
A. versicolar gr.	14.4	Antique bronze	Velvety	Rounded	Moderate
A. flavus	13.84	White -green	Floccose	Rounded	High
A.niger	50.08	Black with White margin	Powdery	Rounded	High
Aspergillus sp.	16.5	Army green with white margin	Downy	Rounded	Moderate
Penicillium funiculosum	32.35	White with brown appearance	Cottony	Rounded	Moderate
Penicillium sp.	28.42	Dark moss green	Velvety	Rounded	Moderate
Penicillium sp.	6.13	Cambridge blue	Velvety	Rounded	Moderate
Absidia corymbifera	58.01	White	Woolly	Rounded	Non sporulating
Cladosporium cladosporiodes	9.39	Bronze yellow	Velvety	Rounded	Moderate
Cladosporium resinae	16.43	Dark brown	Velvety		Moderate
Chrysosporium tropicum	7.58	White cream	Woolly	Rounded	Moderate
Paecilomyces varitii	66.29	Burly wood	Suede-like	Rounded	High
Malbranchea sp.	14.62	Bronze	Velvety	Rounded	Moderate
Mucor sp.	58.01	White	Woolly	Rounded	Non sporulating
Trichoderma sp.	58.01	Acid green	Woolly	Ellipsoid	Very little
Yeast	7.72	Pink	Watery	Rounded	Non sporulating

750 individuals, while the colony of *R. microphyllum* was about 1800 individuals. The relative humidity of the roost sites of *R. hardwickii* and *R. microphyllum* was 80 ± 2 % and 75 ± 15 %, respectively. A fresh polythene sheet (3 x 2 m) was spread on the floor beneath the bat roosts at wee hours and 5 g of bat guano was aseptically collected in sterile vials using forceps. Individuals of *R. hardwickii* and *R. microphyllum* were captured using mist nests, 9 m length, 2 m width and 38 mm mesh size (Avinet, Dryden, USA) which were erected at early morning closest to their roosts for species recognition and also for sample collection. The identification of bat species was carried out based on morphological measurements by following Bates and Harrison (1997). Individual bat was kept in a bat cage for 20 - 30 min for defecation and thereafter released at the site of capture.

Isolation of guanophilic fungus was carried out by suspending 1 g of guano in 9 ml of sterile water to make 10 ml stock suspension. From the stock, 1 ml was taken and tenfold serial dilution was made (Raper et al., 1949; Thom and Raper, 1945). Streptomycin sulphate and tetracycline hydrochloride (8µg/l) were mixed as antibacterial agents with the potato dextrose agar media (Hi-Media) for fungal culture. The serially diluted samples were inoculated onto the culture plates and incubated at 28°C for 7-9 days. The colonies were extirpated and purified in PDA media at 25°C for 7 days. The fungal samples were carefully collected from the inoculation plates and mounted on the aluminum stubs using double side carbon adhesive tapes and kept overnight in desiccators. 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) between 5 and 15 kV at different magnifications.

RESULTS

A total of 16 species of fungi belonging to ten genera were isolated from the guano samples of *R. hardwickii* and *R. microphyllum*. Majority of them belonged to ascomycota (13 species), followed by zygomycota (2 species) and a yeast species (Table 1). Fungal species such as *Aspergillus versicolar gr.*, *A. flavus, A. niger*, *Absidia corymbifera*, *Paecilomyces varitii, Cladosporium cladosporiodes, C. resinae, Chrysosporium tropicum* and *Penicillium funiculosum* were isolated from the guano samples of *R. hardwickii*. In addition, two species of genus *Penicillium*, and one species of each of the genera *Aspergillus, Malbranchea, Trichoderma and yeast* were also isolated from the guano samples of *R. hardwickii*. The guano samples of *R. microphyllum* which collected from Godavari cave temple at Chitrakoot had only *Absidia corymbifera* and sterile mycelia.

The colony of *Aspergillus versicolar* was antique bronze, rounded and velvety (Figure 1A). It attained a diameter of 14.4 mm on seventh day (Table 1). The hyphae boar chains of rough conidia on terminal ends (Figure 2A). The colony of *A. flavus* was pale green with white margin, circular and floccose (Figure 1B). The hyphae of *A. flavus* were septate and dichotomously branched. Conidial heads were radiated, uni-and biseriate. The conidium was pale green and

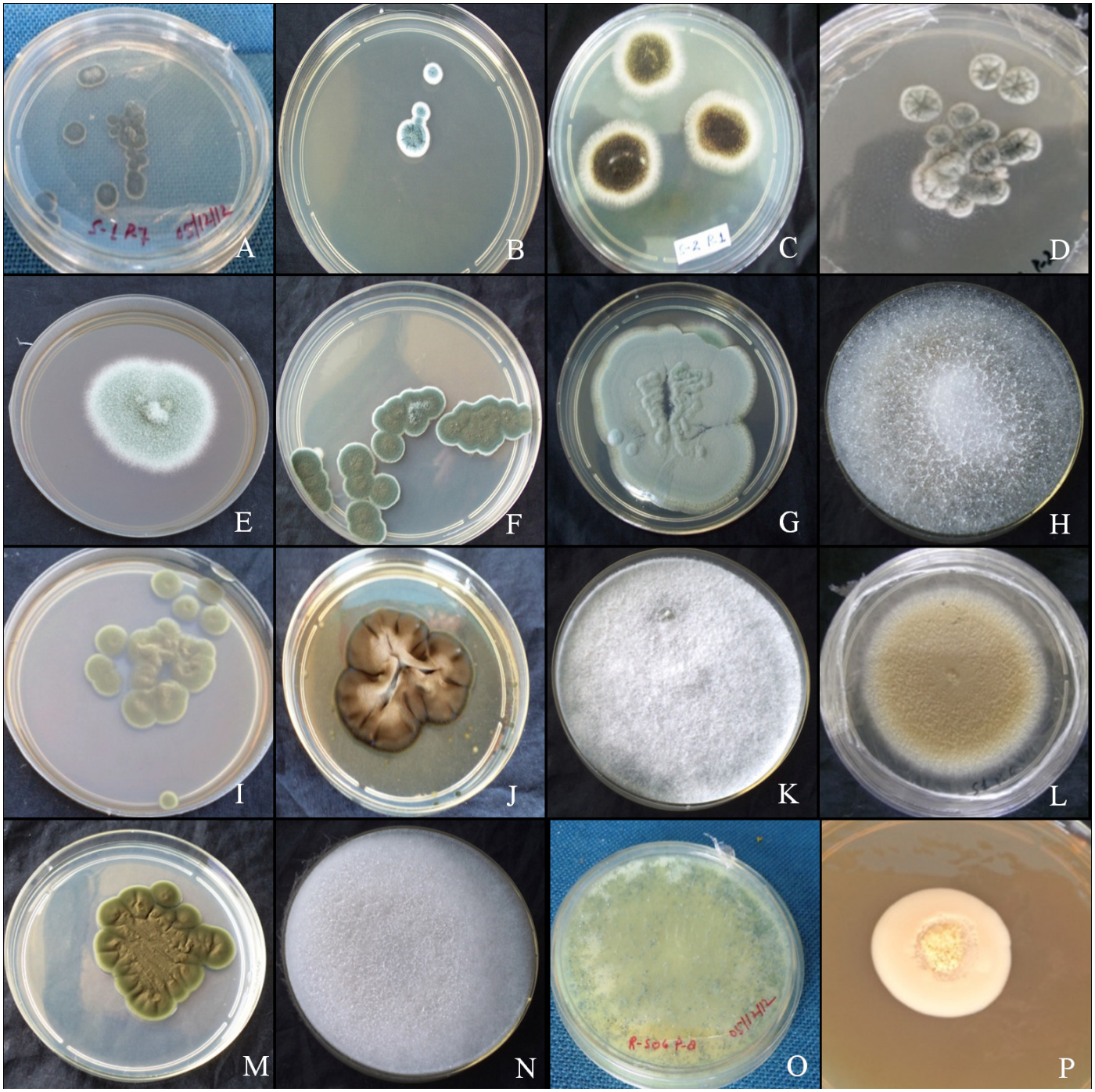

Figure 1: Colony morphology *A. versicolar gr* (**A**), *A. flavus* (**B**) and *A. niger* (**C**). *Aspergillus* sp. (**D**), *Penicillium funiculosum* (**E**), *Penicillium* sp. (**F**), *Penicillium* sp. (**G**), *Absidia corymbifera* (**H**), *Cladosporium cladosporiodes* (**I**), *Cladosporium resinae* (**J**), *Chrysosporium tropicum* (**K**), *Paecilomyces varitii* (**L**), *Malbranchea* sp. (**M**), *Mucor* sp. (**N**), *Trichoderma* sp. (**O**) and *yeast* sp. (**P**)

conspicuously echinulate, smooth to very finely roughened and spherical (Figure 2B). Conidiophores were coarsely roughened and uncolored. Colonies of *A. niger* were isolated from the guano samples of *R. hardwickii* collected from a monument at Khusrubagh, Allahabad and at building roost in Sultanpur. The round and powdery colony with inner black and white margin (Figure 1C) attained 50.08 mm (Table 1). The conidium of *A. niger* was brown to black, very rough and globose (Figure 1C). The conidiophores bore numerous black dot-like spores at the terminal end (Figure 2C). The hyphae were translucent and septate. The conidial heads of *A. niger* were radiated initially and split into columns at maturity. A colony of unknown species belongs to the genus *Aspergillus* was isolated from the guano of *R. hardwickii* collected at Atala mosque, Jaunpur (Figure 2D). The rounded colony was downy; with inner army green and white margin attained 16.5 mm at maturity (Figure 1D).

A colony of *Penicillium funiculosum* was isolated from the guano sample collected from the Thar Ganga Ghat, Varanasi. The colony was cottony, white, rounded and attained

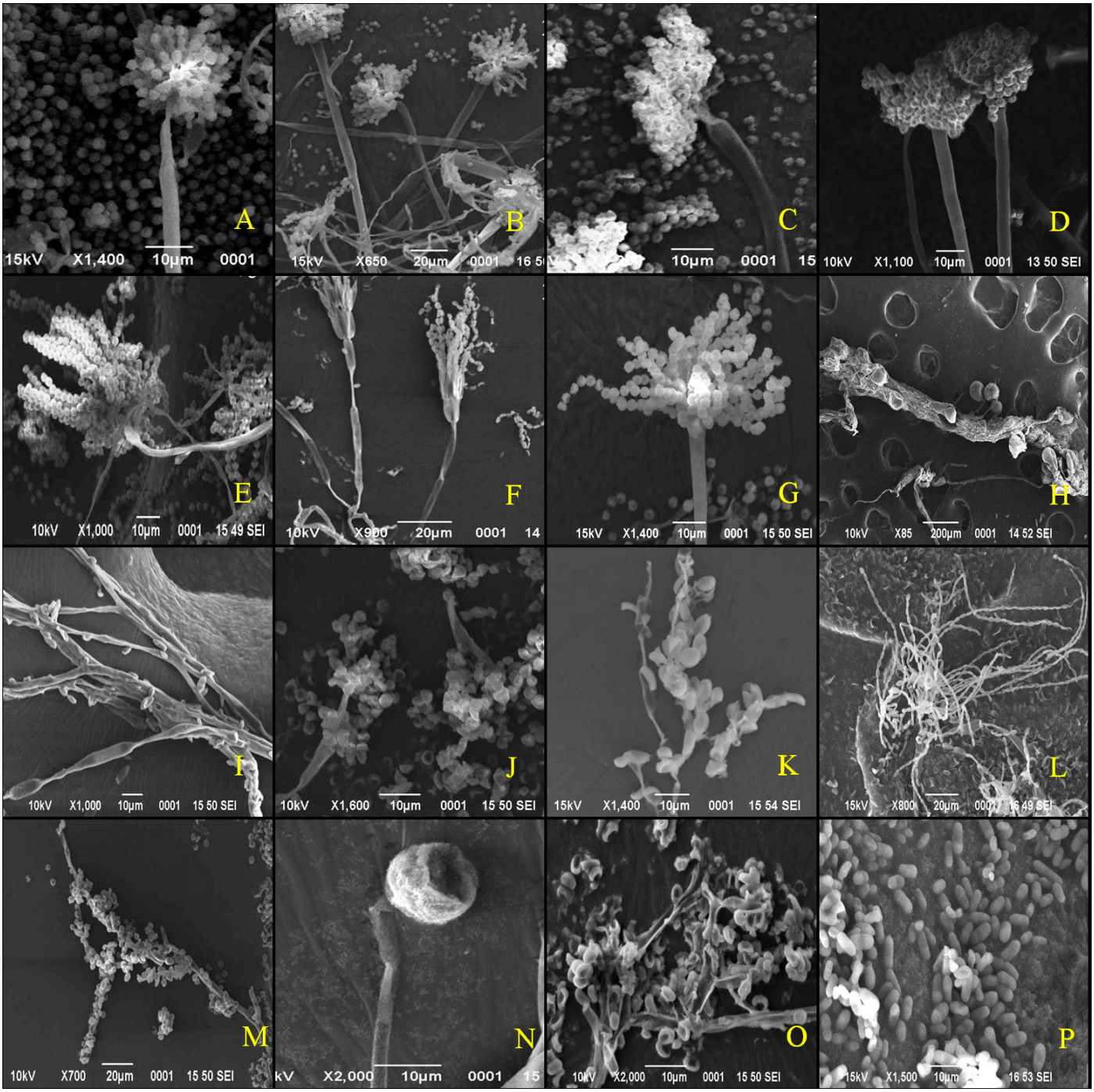

Figure 2: Scanning electron micrographs of *A. versicolar gr* (**A**), *A. flavus* (**B**) and *A. niger* (**C**). *Aspergillus* sp. (**D**), *Penicillium funiculosum* (**E**), *Penicillium* sp. (**F**), *Penicillium* sp. (**G**), *Absidia corymbifera* (**H**), *Cladosporium cladosporiodes* (**I**), *Cladosporium resinae* (**J**), *Chrysosporium tropicum* (**K**), *Paecilomyces varitii* (**L**), *Malbranchea* sp. (**M**), *Mucor* sp. (**N**), *Trichoderma* sp. (**O**) and *yeast* sp. (**P**)

32.35 mm (Figure 1E). A large number of whip-like conidial chains observed at the terminal end of conidiophore with spherical spores (Figure 2E). In addition, two isolates of *Penicillium* were collected from the guano of *R. hardwickii*. A dark moss green, velvety and round colony was isolated from the guano samples collected from Diyara, Sultanpur attained 28.42 mm (Figure 1F). The conidiophores consist long and oval spores at the terminal end (Figure 2F). Another rounded and velvety colony with Cambridge blue was isolated from the sample collected from Khusrubagh fort, Allahabad (Figure 1G). The matured colony attained 6.13 mm. The conidiophore was branched and bore chains of conidia at the terminal end (Figure 2G).

Absidia corymbifera was isolated from the guano samples of *R. hardwickii* collected at Khusrubagh fort, Allahabad and *R. micorphyllum* samples from Godavari cave temple, Chitrakoot. The colony of *A. corymbifera* was white, wooly, rounded and attained 37.93 mm at maturity (Figure 1H). The branched conidiophores bore few numbers of conidia (Figure 2H). Two species of *Cladosporium* were

isolated from the guano samples of *R. hardwickii* (Table 1). *Cladosporium cladosporoides* was isolated from a roost at the riverbank of Ganga, Varanasi and *C. resinae* was isolated from a monument at Sultanpur. The colony of *C. cladosporoides* was bronze yellow, velvety, rounded and attained 9.39 mm (Figure 1I). The septate hyphae had spherical conidia. The conidia were sparsely attached on the hyphae (Figure 2I). The colony of *C. resinae* was moderately sporulated, dark brown, velvety and attained 16.43 mm (Figure 1J). The first conidium was developed in to ramoconidia with three protuberant scars and conidiophores arose laterally from vegetative hyphae (Figure 2J). *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 (Figure 1K). The thin walled hyphae were hyaline, branched and divided by septa. The barrel-shaped conidium was solitary, terminal and stalked (Figure 2K). The colony of *P. varitii* was wooly, rounded, burly wood colour and attained a diameter 66.29 mm (Figure 1L). The hyphae were septate, branched and bore chains of spores (Figure 2L).

In addition, one species belongs to each of genera *Malbranchea*, *Mucor*, *Trichoderma* and yeast was isolated. The colonies of *Malbranchea* sp. and *Mucor* sp. were 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 (Figure 1M). The branched and segmented hyphae bore spores (Figure 2M). The colony of *Mucor* sp. was white, wooly, rounded and attained 58.01 mm (Figure 1N). The hyphae were hyaline with terminal conidia (Figure 2N). The colony of *Trichoderma* sp was acid green, wooly and attained 58.01 mm (Figure 1O). The hyphae were highly branched and bore spores (Figure 2O). The yeast colony was pink, wooly, rounded, non-sporulating and attained 7.72 mm with capsule-like individual yeast (Figure 1P, 2P). 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.

DISCUSSION

In the present study, a total of 16 species of ecologically and medically important fungi were isolated from the guano samples of *R. hardwickii* and *R. microphyllum*. Among the guanophilic fungi, species belong to genus *Aspergillus* represented more than other genus. It shows that the species belong to *Aspergillus* distributed widely in the guano and various roost sites of rhinopomatid bats. 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). However, *A. niger* has been consider as safe by the US Food and Drug Administration. *Aspergillus niger* produces many industrial important enzymes like amylase, amyloglucosidase, cellulases, glucoamylase, lactase, invertase, pectinase (Gautam et al., 2011). *Aspergillus versicolor* found in the guano of *R. hardwickii* 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). It produces sterigmatocystin, a mycotoxin that is a precursor of aflatoxin B1 (Mills and Abramson, 1986; Tuomi et al., 2000; Nielsen, 2003; Veršilovskis and Saeger, 2010). *Aspergillus flavus* causes chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation. It also causes otitis, cutaneous aspergillosis, pulmonary and systemic infections in immunocompromised patients.

Results of the present study showed the wide distribution of filamentous fungus *Penicillium* in the guano of rhinopomatid bats. Among three species of *Penicillium*, *P. funiculosum* has industrial applications as it involves in cellulose production (Roberto et al., 2013). *Penicillium funiculosum* is able 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). The occurrence of *P. funiculosum* in bat guano attributes that the insectivorous bat *R. hardwickii* consumes insects or insect pests which are reservoirs of *P. funiculosum*. *Absidia corymbifera* isolated from *R. hardwickii* and *R. microphyllum* is opportunistic mycoses. It causes zygomycosis in immunocompetent hosts (Hagensee et al., 1994; Ribes et al., 2000). *Absidia corymbifera* is most commonly reported as an animal pathogen and causes mycotic abortion in cows (Knudtson and Kirkbride, 1992). *Cladosporium cladosporioides* listed as occasional agent of phaeohyphomycosis was isolated from the guano of *R. hardwickii*. 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).

Chrysosporium tropicum is a potent keratinophilic fungus isolated from the guano of *R. hardwickii*. It decomposes the most abundant and highly stable animal protein keratin (Avasan et al., 2011). As *R. hardwickii* feeds on orthop-

teran, dictyopteran, lepidopteran, hymanopteran, coleopteran and dipteran insects, the occurrence of *C. tropicum* in its guano is ecologically important for decomposing the insect keratin. *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 et al., 2009). *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). The occurrence of *Malbranchea* sp. in the bat guano was apparently due to contamination. *Mucor* isolated from the guano of *R. hardwickii* causes opportunistic infections known as zygomycosis (Larone et al., 1995; Stewart et al., 1999), which includes infections in mucous membranes, nasal passages and sinuses, eyes, lungs, skin, and brain, as well as renal and pulmonary infections and septic arthritis. *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 two insectivorous bats and their active role on ecosystem and human health.

AUTHORS' CONTRIBUTION

Both authors contributed equally.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of India.

ACKNOWLEDGMENTS

We thank the University Science Instrumentation Centre for extending scanning electron microscope facility and the Archaeological Survey of India for permitting us in the monuments to collect guano samples. The financial assistance of Science and Engineering Research Board, Department of Science and Technology, New Delhi through a research project (No. SB/EMEQ-009/2013) to VE is acknowledged. PKM is a UGC-RGN fellowship holder.

REFERENCES

- 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.
- Ajello L (1986). Hyalohyphomycosis and phaeohyphomycosis: two global disease entities of public health importance. Eur. J. Epidemiol. 2: 243 - 251. http://dx.doi.org/10.1007/BF00419488
- 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.
- 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. http://dx.doi.org/10.1073/pnas.1000454107
- Anderson B, Frisvad JC, Sondergaard I, Rasmussen IB, Larsen LS (2011). Associations between fungal species and water-damaged building materials. Appl. Environ. Microbiol. 77: 4180 - 4188. http://dx.doi.org/10.1128/AEM.02513-10
- 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.
- 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 captured in Peruvian Amazon basin region. Am. J. Trop. Med. Hyg. 63: 255 - 258.
- Bates PJJ, Harrison DL (1997). Bats of the Indian Subcontinent. Harrison Zoological Museum Press, England.
- Bunnell JE, Hice CL, Watts DM, Montrueil V, Tesh RB, Vinetz JM (2000). Detection of pathogenic *Leptospira* spp. infections among mammals captured in Peruvian Amazon basin region. Am. J. Trop. Med. Hyg. 63: 255 - 258.
- 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. http://dx.doi.org/10.1007/s10295-009-0656-2
- Durakovic S, Gallic J, Pajnovic P (1989). Toxic and cancer metabolites of moulds in food and fooder. Hrana. I. Ishrana. 30: 71 - 100.
- Edmondson DA, Barrios CS, Brasel TL, Straus DC, Kurup VP, Fink JN (2009). Immune response among patients exposed to molds. Int. J. Mol. Sci. 10: 5471 - 5484. http://dx.doi.org/10.3390/ijms10125471
- 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. http://dx.doi.org/10.1128/AEM.68.8.3886-3890.2002
- Garcia-Hermoso D, Mathoulin-Pelissier S, Couprie B, Ronin O, Dupont B, Dromer F (1997). DNA typing suggests pigeon droppings as a source of pathogenic *Cryptococcus neoformans* serotype D. J. Clin. Microbiol. 35:2683-2685.
- Gautam AK, Sharma S, Avasthi S, Bhadauria R (2011). Diversity, Pathogenicity and toxicology of *A. niger*: An important spoilage fungi. Res. J. Microbiol. 6: 270 - 280. http://dx.doi.org/10.3923/jm.2011.270.280
- 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. http://dx.doi.org/10.1093/clinids/19.3.402

- Hendey NI (1964). Some observations on *Cladosporium resinae* as a fuel contaminant, and its possible role in the corrosion of aluminium alloy fuel tanks. Trans. Br. Mycol. Soc. 47: 467 - 475. http://dx.doi.org/10.1016/S0007-1536(64)80024-3
- Hill JE (1977). A review of the Rhinopomatidae (Mammalia: Chiroptera). Bull. Br. Mus. Nat. Hist. Zool. 32: 29 - 43. http://dx.doi.org/10.1103/PhysRevA.15.599
- Houbraken J, Varga J, Rico-Munoz E, Johnson S, Samson R (2008). Sexual reproduction as the cause of heat resistance in the food spoilage fungus *Byssochlamys spectabilis* (anamorph *Paecilomyces variotii*). Appl. Environ. Microbiol. 74: 1613 - 1619. http://dx.doi.org/10.1128/AEM.01761-07
- Kis-Papo T, Kirzhner V, Wasser SP, Nevo E (2003). Evolution of genomic diversity and sex at extreme environments: fungal life under hypersaline Dead Sea stress. PNAS, USA. 100: 14970 - 14975. http://dx.doi.org/10.1073/pnas.2036284100
- Knudtson WU, Kirkbride CA (1992). Fungi associated with bovine abortion in the northern plains states (USA). J. Vet. Diagn. Invest. 4: 181 - 185. http://dx.doi.org/10.1177/104063879200400211
- Larone DH (1995). Medically important fungi. ASM Press. Washington.
- Lim TK, Rohrbach KG (1980). Role of *Penicillium funiculosum* strains in the development of pineapple fruit diseases. Phytopath. 70: 663 - 665. http://dx.doi.org/10.1094/Phyto-70-663
- Matsumoto T, Ajello L, Matsuda T, Szaniszlo PJ, Walsh TJ (1994). Developments in hyalohyphomycosis and phaeohyphomycosis. J. Med. Vet. Mycol. 32: 329 - 349. http://dx.doi.org/10.1080/02681219480000951
- Mattsson R, Haemig PD, Olsen B (1999). Feral pigeons as carriers of *Cryptococcus laurentii*, *Cryptococcus uniguttulatus* and *Debaryomyces hansenii*. Med. Mycol. 37: 367 - 369. http://dx.doi.org/10.1046/j.1365-280X.1999.00241.x
- Mbata TI (2008). Isolation of fungi in hyper saline Dead Sea water. Sudanese J. Publ. Health. 3: 170 - 172.
- Mills JT, Abramson D (1986). Production of sterigmatocystin by isolates of *Aspergillus versicolor* from western Canadian stored barley and rapeseed/canola. Can. J. Plant. Pathol. 82: 151 - 153. http://dx.doi.org/10.1080/07060668609501819
- Moreno G, Arenas R (2010). Other fungi causing onychomycosis. Clin. Dermatol. 28: 160 - 163. http://dx.doi.org/10.1016/j.clindermatol.2009.12.009
- Nielsen KF (2003). Mycotoxin production by indoor molds. Fungal G. Biol. 39: 103 - 117. http://dx.doi.org/10.1016/S1087-1845(03)00026-4
- 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. http://dx.doi.org/10.1080/02652030802366090
- Northup DE, Lavoie K, Mallory L (1997). Microbes in caves. Nat. Speleol. Soc. News. 55: 11.
- 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. http://dx.doi.org/10.1007/s11046-005-6871-0
- Pitt JI, Hocking AD (2009). Fungi and food spoilage. Springer, Berlin, Germany. http://dx.doi.org/10.1007/978-0-387-92207-2
- Rai V, Mehrotra S (2005) Toxic contaminants in herbal drugs. Environ. News Arch. 11:1-3.
- Rao M, Gaikwad S, Mishra C, Deshpande V (1988). Induction and catabolite repression of cellulase in *Penicillium funiculosum*. Appl. Biochem. Biotech. 19: 129 - 137. http://dx.doi.org/10.1007/BF02921478
- Raper KB, Thom C, Fennel DI (1949). A Manual of penicillia. Baltimore Press Inc. USA. Pp. 63.
- Ribes JA, Vanover-Sams CL, Baker DJ (2000). Zygomycetes in human disease. Clin. Microbiol. Rev. 13: 236 - 301. http://dx.doi.org/10.1128/CMR.13.2.236-301.2000
- 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. http://dx.doi.org/10.1016/j.jbiotec.2012.10.014
- Salle V, Lecuyer E, Chouaki FX, Lescure A, Smail A, Vaidie C, Dayen JL, Schmit JP, Ducroix, Douadi Y (2005). *Paecilomyces variotii* fungemia in a patient with multiple myeloma: case report and literature review. J. Infect. 51:e93-e95 http://dx.doi.org/10.1016/j.jinf.2004.09.006
- Samson RA (1974). *Paecilomyces* and some allied hyphomycetes. Stud. Mycol. 6:1 - 119.
- 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. http://dx.doi.org/10.1007/s00253-002-1032-6
- Shelton BG, Kirkland KH, Dana W (2002). Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl. Environ. Microbiol. 68: 1743 - 1753. http://dx.doi.org/10.1128/AEM.68.4.1743-1753.2002
- 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. http://dx.doi.org/10.1080/j.1365-280X.1999.00221.x
- Thom C, Raper KB (1945). A manual of aspergilla. Baltimore Press Inc. USA. Pp. 45.
- Truckesses MW, Scoot PM (2009). Mycotoxins in botanical and dried fruits: A review. Addit. Cont. 25: 181 - 192. http://dx.doi.org/10.1080/02652030701567459
- Tuomi T, Reijula K, Johnsson T, Hemminki K, Hintikka EL, Llindroos O, Kalso S, Koukila-Kähkölä P, Mussalo-Rauhamaa H, Haahtela T (2000). Mycotoxins in crude building materials from water damaged buildings. Appl. Environ. Microbiol. 66: 1899 - 1904. http://dx.doi.org/10.1128/AEM.66.5.1899-1904.2000
- Veršilovskis A, Saeger SD (2010). Sterigmatocystin: occurrence in foodstuffs and analytical methods - an overview. Mol. Nut. Food. Res. 54: 136 - 147. http://dx.doi.org/10.1002/mnfr.200900345
- Weinding R (1934). Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathology. 24: 1153 - 1179.
- Yamamoto Y, Kohno S, Koga H (1995). Random amplified polymorphic DNA analysis of clinically and environmentally isolated *Cryptococcus neoformans* in Nagasaki. J. Clin. Microbiol. 33: 3328 - 3332.

Advances in Life Sciences 5(9), Print : ISSN 2278-3849, 3649-3654, 2016

Light and Scanning Electron Microscopic Studies on Food Habit Analysis of Insectivorous Bats

PAWAN KUMAR MISRA AND VADAMALAI ELANGOVAN*

Department of Applied Animal Sciences,
Babasaheb Bhimrao Ambedkar University, Vidya Vihar
Raibareli Road, Lucknow-226025
email: elango70@yahoo.com

ABSTRACT

The food habit analysis of eight insectivorous bats such as *Rhinopoma hardwickii*, *R. microphyllum*, *Scotophilus heathii*, *S. kuhlii*, *Pipistrellus coromandra*, *Tophozous nudiventris*, *Megaderma lyra* and *Hipposideros fulvus* were studied using light and scanning electron miscroscopes. The bat guano was collected from unused buildings, historical monuments and caves from 15 districts of Uttar Pradesh. The pellet analysis revealed that the insectivorous bats fed on nocturnal insects belong to orders Coleoptera, Hymenoptera, Odonata, Hemiptera, Neuroptera, Lepidoptera and Diptera over the study period. The insect remnants such as legs, wings, antennae and elytra were commonly observed in the faecal pellets. The results lead to the conclusion that different insectivorous bats selectively fed on nocturnal insects, possibly agricultural pests, thus the conservation of insectivorous bats would facilitate to control agricultural pests and maintain a balanced ecosystem.

Key words *Bat guano*, *insectivorous bat*, *insect pest*, *biological control*

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 insectivorous bats need to feed a large amount of insects every night to meet the energy demands for their high powered flight (Altringham, 1996). Therefore, the insectivorous bats are called voracious feeders of nocturnal insects including many crop and forest pests. The insectivorous bats mainly prey on moths, flies, midges, mosquitoes, beetles, bugs, and other insects that cause massive alleviation to crops. However, it is unclear whether they select precise insect species as prey or consume solely those species occurring in the greatest abundance remains unclear (Buchler, 1976; Fenton and Morris, 1976; Anthony and Kunz, 1977). Sophia (2010) reported that the foraging time of a bat and the activity period of insect pests consumed by the bat species coincide. Exclusion of bats leads to a distinct increase in arthropod herbivory, emphasizing the prominent role of vertebrate predators in controlling arthropods.

Whitaker (1978) purposed that the identification of prey consumed by insectivorous bats, at least, order level possible because most bats do not feed on diverse categories of insects at the solitary period. The state Uttar Pradesh has a rich diversity of insectivorous bats, e.g. *Rhinopoma hardwickii*, *R. microphyllum*, *Scotophilus heathii*, *S. kuhlii*, *Pipistrellus. coromandra*, *Taphozous nudiventris*, *Megaderma lyra* and *Hipposideros fulvus*. However, the food and feeding habits of insectivorous were not well studied. The present study was aimed to fulfill the lacuna on food and feeding habits of insectivorous 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. nudiventris*, *M. lyra* and *H. fulvus* were collected from their roosting sites by spreading 2 x 2 m polythene sheet beneath the roosts. Guano samples were collected in 5 ml sample vials and kept in the

Table 1. Roost type and roost location of insectivorous bats.

S. No.	Bat species	Site of sample collection	Roost type
1	*R. hardwickii*	Khusrubagh, Allahabad (25.43°N 81.93°E)	Old Monuments
		Thar Ganga Ghat, Varanasi (25.28° N, 82.96° E)	Abandoned building
		Atala Mosque, Jaunpur (25.75° N, 82.69° E)	Historical Monument
		Chunar, Mirzapur (25.13° N, 82.90° E)	Historical Monument
		Sidharth Nagar (27.30° N, 83.09° E)	Historical Monument
		Kunda, Pratapgarh (25.72° N, 81.52° E)	Abandoned building
2	*R. microphyllum*	Chunar, Mirzapur (25.13° N, 82.90° E)	Historical Monument
		Purwa, Unnao (26.47° N, 80.78° E)	Abandoned building
3	*S. heathii*	Ayodhya, Faizabad (26.80° N, 82.20° E)	Historical Monument
		Hardoi (27.42° N, 80.12° E)	Abandoned building
4	*S. kuhlii*	Atala Mosque, Jaunpur (25.75° N, 82.69° E)	Historical Monument
		Bangarmau, Hardoi (27.42° N, 80.12° E)	Abandoned building
		Bachhrawa, Lucknow (26.80° N, 80.90° E)	Abandoned building
5	*P. coromandra*	Mirzapur (25.15° N, 82.60° E)	Abandoned building
6	*T. nudiventris*	Jhansi Fort, Jhansi (25.44° N, 78.56° E)	Historical Monument
		Mallawa, Hardoi (27.42° N, 80.12° E)	Abandoned building
		Purwa, Unnao (26.47° N, 80.78° E)	Abandoned building
7	*M. lyra*	Bangar Mau Hardoi (27.42° N, 80.12° E)	Abandoned building
		Puraini, Bahraich (27.59° N, 81.59° E)	Abandoned building
		Diyara, Sultanpur (26.25° N, 82.00° E)	Abandoned building
		Chunar, Mirzapur (25.15° N, 82.60° E)	Abandoned building
8	*H. fulvus*	Bara Imambara, Lucknow (26.86° N, 80.91° E)	Historical Monuments

refrigerator (at 4 °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 such as legs, wings, antennae and elytra 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 4ÚC, 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 ÚC and washed in 0.1 M phosphate buffer thrice each at 15 min interval at 4ÚC. The samples were dehydrated in ascending series of acetone followed by dry acetone. Samples were mounted on 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 AND DISCUSSION

A total of eight species of insectivorous bats such as *R. hardwickii*, *R. microphyllum*, *S. heathii*,

Table 2. The food choice different insectivorous bats. '+' and '-' indicate the presence and absence of insect remnants of respective insect orders, respectively.

Bat species	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	+	-	-	+	+	-	-	-

S. kuhlii, *P. coromandra*, *T. nudiventris*, *M. lyra* and *H. fulvus* were found in 15 districts of Uttar Pradesh, India (Table 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

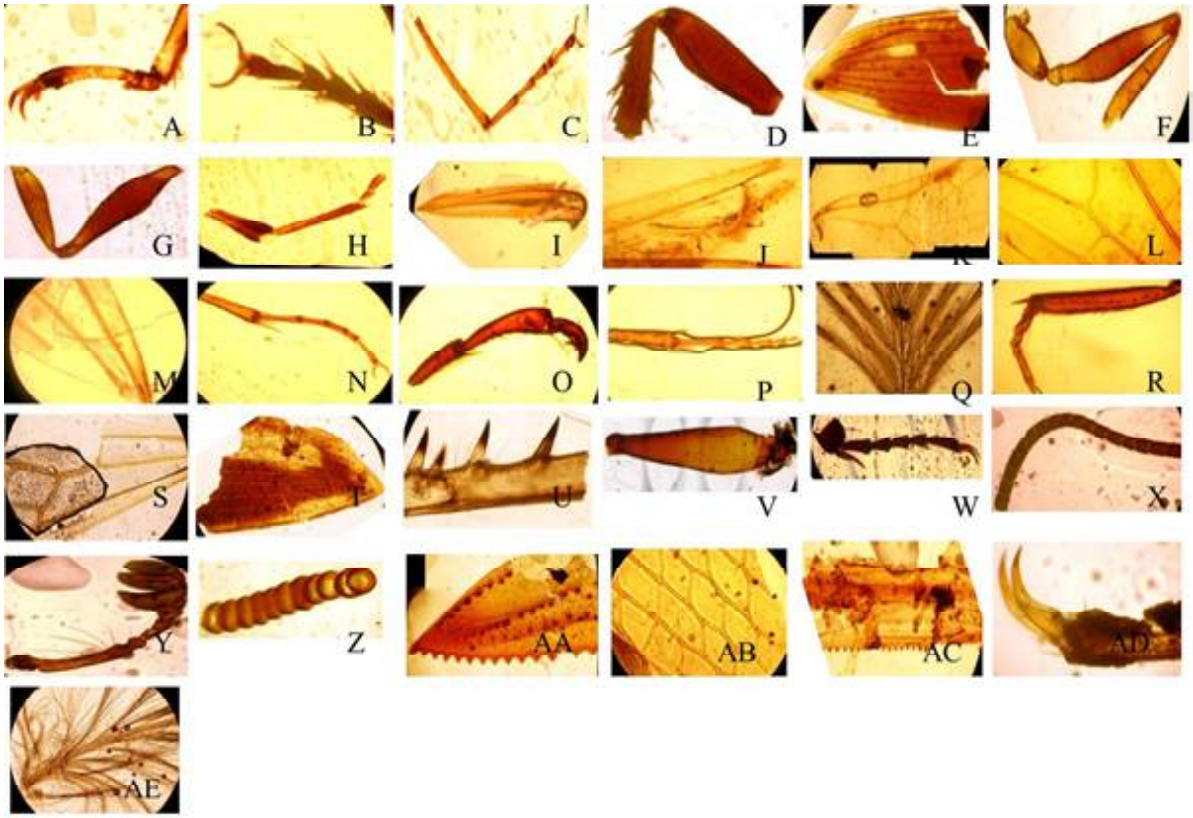

Fig. 1. Light microscope images of food remnants: Legs of Hymenoptera (A, B and C), Coleoptera (D), Elytra of Coleoptera (E), Odonata (F and G) and Orthoptera (H and I), Wing of Neuroptera (J and K), wing of Hymenoptera (L and M), Coleoptera (N), leg of Orthoptera (O), legs of Diptera (P), leg of Odonat (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) feather (AE).

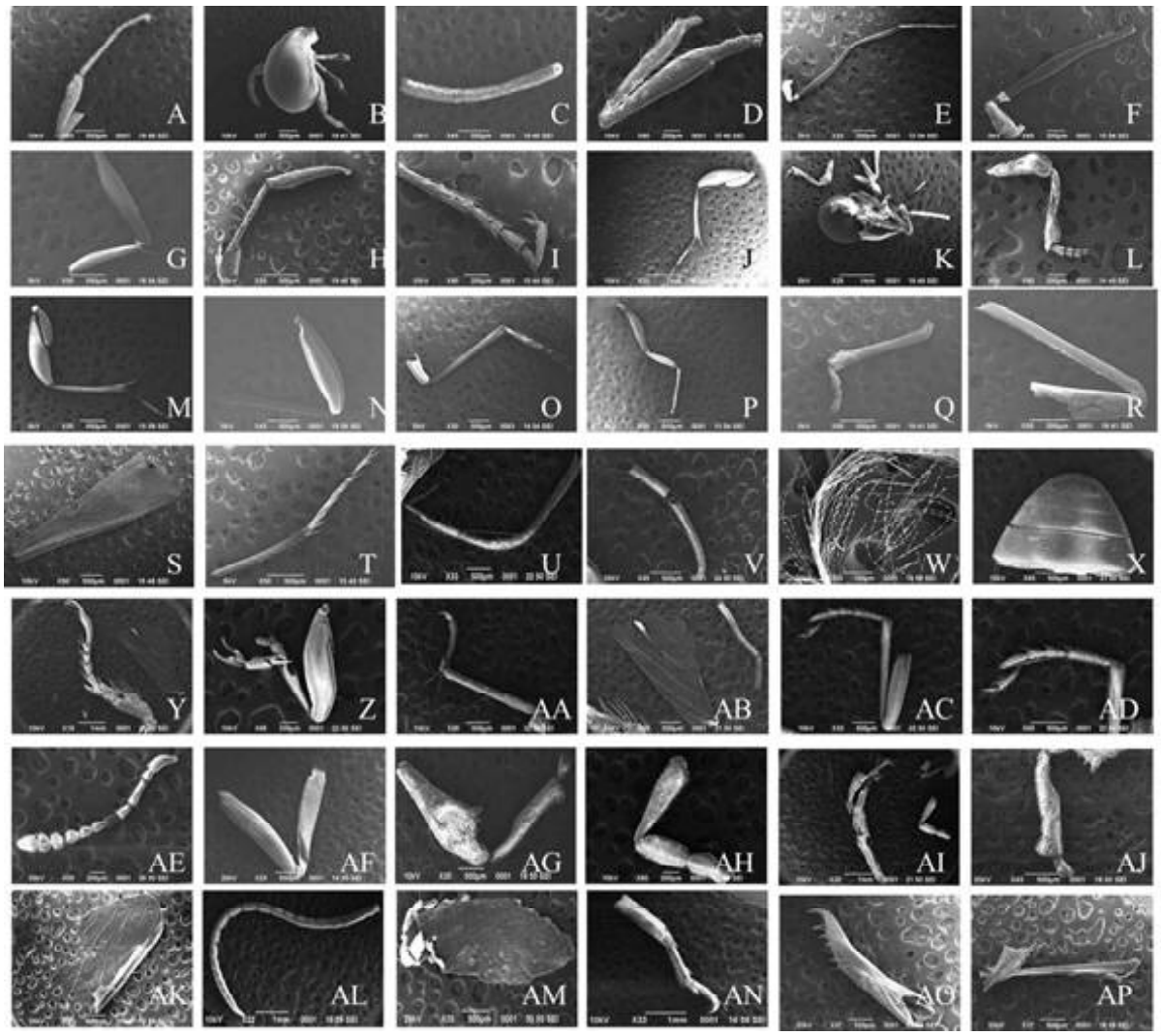

Fig. 2. Scanning Electron Microscope images of food remnants: Leg of Hymenoptera (A), Beetle (B), antenna of Coleoptera (C), legs of Hymenoptera (D and E), leg of Coleoptera (F), leg of Hymenoptera (G), leg of Coleoptera (H – J), Whole body of Coleoptera (K), leg of Coleoptera (L – N), leg of Diptera (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).

order Coleoptera, Hymenoptera, Odonata, Hemiptera, Neuroptera, Lepidoptera and Diptera (Table 2).

The guano samples of *R. hardwickii* were collected from Allahabad, Varanasi, Jaunpur, Mirzapur and Pratapgarh districts of Uttar Pradesh (Table 1). The guano samples of *R. hardwickii* had the body parts of Coleoptera, Hymenoptera, Odonata and Neuroptera insect orders (Table 2). The undigested leg parts of Hymenoptera (Fig. 1 A, B and C), Coleoptera (Fig. 1 D), Odonata (Fig. 1 F and G) and Orthoptera (Fig. 1 H and I) were observed in the guano of *R. hardwickii*. Further, the guano of *R. hardwickii* had wings of Neuroptera (Fig. 1 J and K) and elytra of Coleoptera (Fig. 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. 1 L and Fig. 1 M), legs of Diptera (Fig. 1 P), Coleoptera (Fig. 1 N, Fig. 2 F).

Guano samples of *S. heathii* were collected from Purwa, Unnao, Ayodhya, Faizabad and Hardoi districts (Table 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. 2 B), wings of Hymenoptera (Fig. 1 S), elytra (Fig. 1 T) and antenna (Fig. 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 2). In addition, the legs of Hymenoptera (Fig. 1U, Fig. 2 D and G), Orthoptera (Fig. 2 S) and Lepidoptera (Fig. 2 T), Diptera (Fig. 2 Q - Q) and wings of Odonata (Fig. 2 R) were observed. *Pipistrellus coromandra* was collected from Chunar fort, Mirzapur district and found the antennae of coleopteran insects (Fig. 2 U and Fig. 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 2). The remnants include abdominal segments (Fig. 2 X) and legs (Fig. 2 Y, Z) of Coleoptera, legs (Fig. 1V, 2 AA) and wings (Fig. 2 AB) of Orthoptera and legs (Fig. 2 AC, AD) and wings (Fig. 1 W) of Hymenoptera were observed. The guano samples of *M. lyra* were collected from Purwa, Unnao, Hardoi, Bahraich, Sultanpur and Mirzapur (Table 1). In the guano of *M. lyra*, the remnants belong to orders Coleoptera, Hymenoptera andOrthoptera were observed. The antenna (Fig. 1 X – Z, Fig. 2 AE) of Coleoptera and mandible (Fig. 1 AA) of Hymenoptera (Fig. 1 AE) were observed. The guano of *H. fulvus* was collected from Bara Imambara, Lucknow (Table 2). The remnants include legs of Hymenoptera (Fig. 2 AG - AJ), Coleoptera (Fig. 2 AK) and Orthoptera (Fig. 2 AO and AP), wings (Fig. 2 AK), antenna (Fig. 2 AL) and elytra (Fig. 2 AM) of Coleoptera were observed in the guano of *H. fulvus*.

The results of present study revealed that the insectivorous bats commonly feed on the insects belong to orders such as Coleoptera, Hymenoptera, Odonata, Hemiptera, Neuroptera, Lepidoptera and Diptera. The insects belong to the orders Coleoptera, Lepidoptera, Homoptera and Hemiptera are the major agricultural pests (Oliveira 2005) and the insectivorous bats food remnants showed that they feed predominantly on insect pests. The body parts of hymenopteran insects (flying ants) observed in the guano of all bat species except *P. coromandra* suggests the wide distribution of flying ants and prefered diet of all insectivorus bats. The results show that *S. kuhlii* predate the insects of at least six insect orders such as Coleoptera, Lepidoptera, Diptera, Hymenoptera, Orthoptera and Odonata. and predation on few insect orders were already reported (Srinivasulu *et al*., 2010).*Megaderma lyra* prefers to capture prey such as large insects and small vertebrates (Advani, 1981; Habersetzer, 1983) from the ground and water surfaces. In this study, insect belonged to Coleoptera, Hymenoptera and Orthoptera were recorded. Our results lead to the conclusion that various species of insectivorous bats fed selectively on insects belonged to certain insect orders and mainly nocturnal insect pests. Therefore, the insectivorous bats could be appropriate bio-controlling agents to keep the nocturnal insect pests in an ecologically balanced condition. Thus, the study urges to conserve the insectivorous bats to keep the nocturnal insect pests under control.

LITERATURE CITED

Anthony, E.I.P. and Kunz, T.K. 1977. Feeding strategies of little brown bat, *Myotis lucifugus* in Southern New Hampshire. *Ecology*, **58**: 775-786.

Advani, R. 1981. Seasonal fluctuations in the feeding ecology of the Indian false vampire *Megaderma lyra* (Chiroptera: Megadermatidae) in Rajasthan. *Zeits. Saug,* **46** (2): 90-93.

Altringham, D. 1996. Bats: biology and behaviour. Oxford University Press, New York.

Buchler, E. R. 1976. Prey selection by *Myotis lucifugus* (Chiroptera: Vespertilionidae). *American Naturalist* **110**: 619-628.

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

Fenton, M.B. and Morris, G. K. 1976. Opportunistic feeding

by desert bats (*Myotis sp.*). *Canadian J. Zool*, **54**: 526-530.

Habersetzer, J. and Storch, G. 1989. Ecology and echolocation of the Eocene Messel bats. In: Hana´ k V, Hora´c¡ek I and Gaisler J, eds. European bat research 1987. Prague: Charles University Press, 213 - 133.

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

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

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

Whitaker, J. O. 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, J. O. JR. 1995. Food of the big brown bat, *Eptesicus fuscus*, from maternity colonies in Indiana and Illinois. *American Midland Naturalist*, **134**: 346-360.

Received on 23-04-2016 *Accepted on 28-04-2016*