Distribution, occurrence and natural invertebrate hosts of indigenous entomopathogenic fungi of Central India

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Abstract We report here that, during periodical surveys of insects inhabiting diverse habitats for the collection of entomopathogenic fungi; a large number of isolates were recovered belonging to seven species, from various regions of Madhya Pradesh and Chhattisgarh forest areas and agricultural fields. The most common entomopathogenic fungi such as *Beauveria bassiana*, *Nomuraea rileyi*, *Paecilomyces farinosus* and *Paecilomyces fumosoroseus* were found to infect various insect hosts species naturally viz. *Hyblaea puera*, *Eutectona machaeralis*, *Diachrysia orichalcea*, *Spodoptera litura*, and few new insect hosts of these fungal pathogens among Indian insect population were collected for the first time from Central India, such as beetles of *Agrilus* species, hairy caterpillars of *Lymantria* species. The isolation, identification, maintenance and pathogenicity assay of these isolates was performed prior to deposition in culture collection center.

Keywords Biodiversity · Central India · Entomopathogenic fungus · Fungal germplasm collection · Invertebrate hosts

Introduction

The entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin, *Nomuraea rileyi* (Farlow) Samson, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *Paecilomyces farinosus* (Dickson and Fries) Brown and Smith are known to be pathogenic to numerous insect species. These fungi have been considered as a source of biocontrol agents [1] and more recently they have also been considered to be a rich source of natural bioactive compounds [2]. The occurrence of entomopathogenic fungi in India has been reported by many workers [3–11]. These findings indicate that these fungi act as mortality factors in Indian insect populations. However, the abundance and biodiversity of these fungi is poorly known, especially in Central India.

Since very little is known about the extent of their distribution in different habitats of Central India and no previous work on taxonomy with biodiversity studies has been reported. The aim of this research study was to examine this component from the standpoint of the tropics, where fungi are a major component of biodiversity. The isolation of different species of indigenous entomopathogenic fungi from insect populations of Central India will enhance its use as a biological control agent for sustained control of insect pests.

For the collection of cadavers, infected with fungi, local and regional faunal surveys were conducted in various portions of Madhya Pradesh state, including some parts of Chhattisgarh state of Central India, both for the purpose of recovering potentially useful isolates and gaining an insight into the ecology of the indigenous entomopathogenic fungi of Central India. An understanding of the factors governing

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the natural occurrence and abundance of these fungi is of prime importance in formulating a rational approach to their utilization as biological insecticides.

Materials and methods

Survey for collection of cadavers

Collection of insect cadavers was made to (a) identify the insect host and its etiologic agent of disease; and (b) preserve live fungal material for isolation and culturing on artificial media. Systematic periodical surveys to collect entomopathogenic fungi from Central India were started from the month of September 1999 during the dry season after monsoon. Each year from September to February (1999 to 2005) an extensive survey coincided with active period that is until the availability of various insect pests in forest areas and agricultural fields.

All teak plantation and forest areas with heavy infestations of teak defoliator, *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae) and skeletonizer, *Eutectona machaeralis* (Walker) (Lepidoptera: Pyralidae) were surveyed. Most of agricultural fields surveyed, received normal maintenance (i.e. weeding, low level fertilization, adequate irrigation), but were not treated with insecticides or fungicides. Detection of entomopathogenic fungi in insect populations was achieved by direct methods [12]. Cadavers were collected throughout the forest areas and agricultural fields at sites nearly 0.1–0.5 km away from the roadways. Cadaver collection was accomplished mainly through sight inspection. About a hectare of the area at each site (84 sites) was selected for the observations. The vegetation, soil, leaf litter, under stones and tree bark within this area were searched for 1–2 h for insect cadavers and about 2–12 cadavers were collected from each site.

The lethargic insects/dead cadavers adhered to the leaves, bark and soil with or without external signs of mycosis were collected with a piece of substrate to which they adhered. The substrate with attached cadavers was placed in a zip-lock polythene bags whereas cadavers without substrate were kept in sterilized plastic vials with screw caps. The collected material kept in plastic containers was transported promptly to the laboratory. When transported over a long distance, the collections in containers were kept cool by placing them inside coolers maintained below 20°C with ice bags. All the cadavers were stored at 4°C until processing. The microscopic observations of morphological characteristics of fungi *in situ* were made from the superficial scrapings and/or

after isolation in pure culture. Temporary slides were made initially and then permanent slides were prepared [13].

Isolation of entomopathogenic fungi

Mummified insects with evidence of fungal growth were examined under a dissecting microscope and if it was found to be infected with fungi, were recovered frequently onto Malt extract agar medium enriched with 0.5% (w/v) yeast extract (MEAY), 0.1% (w/v) rose bengal and 100 mg/l streptomycin. Isolation of fungi from cadavers with freshly formed external conidia was achieved by taking conidia with a mycological needle and streaked on MEAY plates [14] and incubated at $28 \pm 1^\circ\text{C}$ in a biological oxygen demand (BOD) incubator [15]. For cadavers covered with white mycelia but did not produce conidia, a small quantity of hyphae was scraped with a mycological needle and transferred onto several different types of agar media with antibiotics such as tap water agar, potato dextrose agar (PDA) and MEAY with 100 mg/l streptomycin at pH 6.7 and incubated at $28 \pm 1^\circ\text{C}$, L:D 16:8 h in a BOD incubator. All cultures were examined daily with a stereoscopic microscope. When more than one species of fungus grew on the same plate, they were separated by subculturing. For isolating pure cultures the small colony of fungus was removed from the agar plates by cutting round the tip of mycelia with a dummy cutter objective or cork borer and then the agar block was transferred to fresh desired medium slants in tubes for preservation [14].

Identification of entomopathogenic fungi

The slide culture technique [16] was adopted for the identification of each isolate and permanent mounts, stained (Cotton blue - Lacto phenol) fungal structures were mounted using dinbutyl phthalate in xylene (DPX). Identification was done on the basis of morphotaxonomic characters identified by microscopically inspecting the conidia-forming mycelia for conidiogenous structure and conidial morphology according to available literature [17–19].

Maintenance of fungal cultures

All the cultures were maintained on desired medium. A unique accession was allotted to each isolate, which was never been reassigned if the isolate was later discarded or died. Stocks of these fungal cultures were maintained on agar slants. These tubes were labeled and were held for many months between subcultures on slopes in a refrigerator at 4°C. Inoculums for subsequent studies were always prepared from the first subculture [20].

Pathogenicity assays

Petriplates were inoculated with conidia of each isolate and incubated for 3 weeks at $28 \pm 1^\circ\text{C}$ in a BOD incubator. Conidia were harvested directly by scraping the agar plates. Conidial suspension was made by suspending harvested conidia in 10 ml sterile distilled water with 0.05% Tween-80. The suspension was vortexed in a cyclomixer to produce a homogenous suspension. The viability of these conidia was determined just prior to application by germination test on SMYA coated slides. Conidial suspension of 8.87×10^7 conidia/ml concentration was prepared using Neubauer's hemocytometer [21].

The efficacy of *N. rileyi* isolates was evaluated against laboratory reared larvae of *Diachrysia orichalcea* (Fabricius) (Lepidoptera: Noctuidae) whereas the efficacies of *B. bassiana*, *P. farinosus*, *P. fumosoroseus* isolates were evaluated against laboratory reared larvae of *H. puera* by spraying 10 ml of conidial suspension (8.87×10^7 conidia/ml) using an atomizer. Five replicates of 10 larvae were used in each experiment. Sterile distilled water with 0.05% Tween-80 solution was used as control having a set of 10 larvae each. After the treatment, these larvae were air dried within a laminar air flow cabinet for 30 min, placed in separate transparent disposable plastic petriplates (85 cm) containing moist cotton pad and disinfected tender sprouting leaf pieces of their respective host plant (soybean leaves for *D. orichalcea* and teak leaves for *H. puera*). These petriplates were incubated at $28 \pm 1^\circ\text{C}$, L:D 16:8 h in a BOD incubator [21].

After 24 h, all the larval lots were examined for mortality in each case and when the experimental larvae died, the pieces of leaves were removed, the bodies were air dried for 1 h, and then moistened by placing wet filter paper or wet absorbent cotton to promote mycelial growth and conidial production. Fungus on each cadaver was checked with a microscope to identify whether it was the inoculated fungus or not. Percentage mortality of the 50 larvae inoculated per isolate was calculated, based on the number of insects confirmed with outer growth of the inoculated fungus. Mortality rate was corrected by Abbott's formula [22]. The time mortality response was evaluated by converting percentage mortality to probit mortality [23].

Results

Survey

The surveys for collection of the fungal pathogens of arthropods pests of forest areas and agricultural fields in Central India was undertaken for the evaluation of

occurrence and distribution of indigenous fungi prevalent in Central Indian habitats and for useful programs for the biological management of the insect pests in future. The surveys reported here are some of the first targeted surveys for the study of biodiversity of entomopathogenic fungi in Central India. Each year between September and February, large numbers of cadavers were collected which appeared symptomatic for fungal infection.

A total of 21 surveys were conducted and over 500 cadavers from 84 collection sites representing forest areas (60 sites) and agricultural fields (14 sites) were collected randomly during 1999 to 2005 from different districts of Central India covering many sites through the regions of Central, Eastern and Southern parts of Madhya Pradesh state and Northern and Southern part of Chhattisgarh state. Survey year, district, number of sites surveyed and number of cadavers collected from forest areas and agricultural fields is given in Table 1.

All stages of insects and different types of arthropods were found to be infected with fungi (Fig. 1). Except the cadaver of *H. puera* (o), *E. machaeralis* (g), *D. orichalcea* (j) and *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) (i) shown in Fig. 1 rest all the insect hosts viz. Giant beetle (a), beetle of *Agrilus* species (b), dipteran fly (c), caterpillar of an unidentified butterfly (d), hairy caterpillar of *Lymantria* species (e), stick insect (f), spider

Table 1 Survey year code, district covered, No. of sites (s) surveyed and No. of cadavers (n) collected during survey of two states of Central India

Code	District	(s)	(n) from habitat	
			(f)	(a)
A1	Dindori	5	28	–
A2	Jabalpur	11	55	24
A3	Katni	7	13	28
A4	Mandla	8	50	–
B1	Bastar	7	39	–
B2	Bhopal	3	–	18
B3	Dantewara	10	57	–
B4	Narsimhapur	2	–	14
C1	Balaghat	3	18	–
C2	Shahdol	3	15	–
D1	Koriya	4	26	–
E1	Seoni	5	32	–
E2	Chhindwara	5	28	–
F1	Betul	4	27	–
F2	Hoshangabad	7	40	–

(f) - Forest area; (a) - Agricultural field.

A - 1999–2000; B - 2000–2001; C - 2001–2002; D - 2002–2003;

E - 2003–2004; F - 2004–2005 (September to February).

Fig. 1 Showing various types of natural invertebrate hosts of indigenous entomopathogenic fungi collected during survey in Central India.

- (a) Lateral view of an infected giant beetle (Coleoptera: Scolytidae) with *Paecilomyces farinosus* (FGCC E-21).
- (b) Tiny brilliant green color beetle, *Agrilus* species (Coleoptera: Buprestidae) infected with *Beauveria bassiana* (FGCC E-58).
- (c) Naturally infected Dipteran fly with *Beauveria bassiana* (FGCC E-90). Head and body junctures overlaid with velutinous mycelia and pale to light cream conidia.
- (d) Black hairy caterpillar, *Lymantria* species (Lepidoptera: Lymantriidae) infected with *Beauveria bassiana* (FGCC E-235).
- (e) Caterpillar of an unidentified butterfly infected by *Beauveria bassiana* (FGCC E-248) showing emerging synnemata.
- (f) Stick insect (Phasmida: Phasmatodea) infected with *Paecilomyces fumosoroseus* (FGCC E-23). Head, body and joints of legs and antennae were overlaid with velutinous young mycelia.
- (g) Larva of major teak pest *Eutectona machaeralis* (Lepidoptera: Hyblaeidae) inside its cocoon infected with *Beauveria bassiana* (FGCC E-73) and showing immerging synnemata.
- (h) Spider (Araneae: Arachnida) infected with *Paecilomyces farinosus* (FGCC E-222), whole body covered with mycelial mat.
- (i) Larvae of soybean pest *Spodoptera litura* (Lepidoptera: Noctuidae) infected with *Nomuraea rileyi* (FGCC E-25) showing green sporulation.
- (j) Larva of soybean pest *Diachrysia orichalcea* (Lepidoptera: Noctuidae) infected with *Nomuraea rileyi* (FGCC E-34) showing characteristic death symptom, head upside.
- (k) Grasshopper (Hemiptera) infected with *Beauveria bassiana* (FGCC E-38).
- (l) Root grub infected with *Beauveria bassiana* (FGCC E-24), body junctures overlaid with and pale to light cream color mycelia.
- (m) White grub (Cockchafer grub), *Melolontha* species (Coleoptera: Scarabaeidae) infected with *Beauveria bassiana* (FGCC E-249).
- (n) Ventral view of an infected adult *Hyblaea* (Lepidoptera: Hyblaeidae) with *Beauveria bassiana* (FGCC E-161).
- (o) Larva of *Hyblaea puera* infected with *Beauveria bassiana* (FGCC E-182) showing the insect body was mummified completely.
- (p) Big black ant (Hymenoptera: Formicidae) infected with *Beauveria bassiana* (FGCC E-131).

(h), grasshopper (k), root grub (l), white grub (m) and black ant (p) are the first published report of some new invertebrate hosts of indigenous fungal entomopathogens, collected for the first time from Central India. One isolate of *B. bassiana* was recovered from an adult moth of *H. puer*a (Fig. 1n).

Fungal infected insects shown in Fig. 1 were collected from diverse habitats of Central India like the giant beetle (a), stick insect (f) and the cadaver of *H. puer*a (o) from the teak forest area in Bastar district; beetle of *Agrilus* species (b) from Sal forest area in Koriya district; cadaver of *E. machaeralis* (g) from the teak forest area in Dantewara district of Chhattisgarh state. Similarly the dipteran fly (c) from the forest area in Dindori district; cadaver of black hairy caterpillar (d) and spider (h) from forest area in Balaghat district; unidentified caterpillar of a butterfly (e) and root grub (l) from teak forest area; cadaver of a grasshopper (k) from agricultural field and the cadaver of *S. litura* (i) from the soybean crop field in Narsimhapur; cadaver of *D. orichalcea* (j) from soybean crop field of Jabalpur district; white grub (m) of *Melolontha* species from forest area in Katni district; adult *Hyblaea* (n) from teak forest area in Mandla district and big black ant (p) was collected from the teak forest area in Hoshangabad district of Madhya Pradesh state of Central India.

Following observations were made while collecting the cadavers. After death, the cadavers were found to be desiccated, never flaccid. In soft-bodied insects, the body was covered with fungal mycelium whereas in hard-bodied insects, bands of fungal hyphae grew from intersegmental membranes and mycelial strands grew from the orifices fixing the insect to the substrate. Spores were usually formed on the outer surface of the insect body, but if the environment was dry the fungus was not visible on the insect body surface. Although finding the cadavers was the best way of collecting pathogens, but if none were found in the field, live insects were collected. A small proportion of live insects were found to be infected with at least one of the fungal pathogens.

During our surveys of different sites 246 entomopathogenic fungal isolates belonging to seven species of fungi known to infect and kill insects from lepidopteran, coleopteran and dipteran hosts, collected, represented only by Hyphomycetes. These efforts produced additional isolates and a sizeable bank of fungal material which is presently in long-term storage at the FBIPL (Fungal Biotechnology and Invertebrate Pathology Laboratory) collection center of entomopathogenic fungi located at the Department of Biological Sciences, R.D. University, Jabalpur, Madhya Pradesh, India and these were also deposited time-to-time to the Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH (Institute of Microbial Technology),

Chandigarh, India. Following isolation from Central India, all fungal identifications were verified at our laboratory prior to placement in permanent storage.

From the results of these surveys, it appears that *B. bassiana* is of widespread occurrence in Central India. Most sites, at which *B. bassiana* was detected, were within a few 100 meters of the roadways. It is clearly evident that *B. bassiana* is distributed predominantly along the teak forests and its occurrence was positively correlated with its wide host range. These teak pests were found to be significantly more abundant in natural forest as opposed to the plantation forest. However, there were no significant differences in the abundance of teak pests, where natural forest is adjacent to plantation forest. Prevalence of *N. rileyi* was noticed in populations of soybean looper. The agricultural field sites surveyed for *N. rileyi* were cropped with soybean and gram. The cadavers symptomatic to be infected with *N. rileyi* from these sites were found to be mainly *S. litura* and *D. orichalcea*. All other crops were attacked by a variety of pests in these areas but no fungal infected cadaver was found from these crops.

Morphological study

Insects were considered as fungus infected when they showed indication of mycelial growth on their body surface or were hard and mummified. Isolation of entomopathogenic fungi was usually most successful from insects either in the final stages of disease or very soon after death of the insect host. All the fungal isolates grew and developed saprophytically on favorable nutrient media and they were maintained *in vitro* in the laboratory. Spores growing on cadavers survived at 4°C in the refrigerator as long as they were kept dried and were also being frozen for future use.

The identification of most fungi was necessarily dependent on the observation of microscopic characters. However, many isolates of common entomopathogens, with relatively little effort and time, were easily identified up to the genus level and in some instances, the species such as *B. bassiana* and *N. rileyi* by observation with either the unaided eye or low magnifications from hand lenses or stereo microscopes. Species identifications usually required confirmation of essential microscopic characters. Conidial measurements have been the critical taxonomic character for species differentiation in the entire genera of entomopathogenic fungi. For most deuteromycetes and for non-fastidious fungi from other groups PDA, Sabouraud dextrose agar (SDA) and Sabouraud maltose yeast agar (SMYA) permitted profuse mycelial growth and good sporulation. The optimum temperature for *in vitro* growth and sporulation is usually around 25–28°C.

The following entomopathogenic fungal species were isolated and collected; *B. bassiana*, *N. rileyi*, *M. anisopliae*, *Lecanicillium lecanii* (Zimmerman) Gams and Zare, *P. fumosoroseus*, *P. farinosus*, *Isaria felina* (de Candelolle: Fries) Fries. A small number of nematodes emerging from the living larvae were also noticed but not identified. The frequency distribution of each genus of entomopathogenic fungi prevalent in Central India during the course of this investigation is given in Table 2.

Table 2 Frequency distribution of seven entomopathogenic fungal species isolated in forest and agricultural habitats of Central India ($n = 246$)

S. No.	Name of fungi	Distribution frequency (%)	Total no. of isolates (n)
1	<i>Beauveria bassiana</i>	71.14	175
2	<i>Nomuraea rileyi</i>	13.41	33
3	<i>Paecilomyces farinosus</i>	6.10	15
4	<i>Paecilomyces fumosoroseus</i>	6.10	15
5	<i>Metarhizium anisopliae</i>	2.03	5
6	<i>Lecanicillium lecanii</i>	0.81	2
7	<i>Isaria felina</i>	0.41	1

Pathogenicity assays

Isolates of each fungal species were shown to be pathogenic to their respective host when topically applied to fresh hosts in petriplates and the cadavers supported fungal sporulation. It was noticed that inoculated larvae under study fed normally for some period initially but after 18–24 h the larvae became sluggish, stopped feeding and did not show any response to external stimulus and died. After death, the larvae turned moribund or get stiffed or mummified. Thirty-six h after mummification mycelial growth appeared on the body of larvae. Later on fungus sporulated profusely on the mummified body of the larvae under the influence of high relative humidity, confirming the pathogenic nature of the isolates.

The results of the pathogenicity assays are given in Table 3. The percentage mortality was given in a range due to large number of fungal isolates tested for their pathogenic nature. Percentage mortality was calculated only after the confirmation supported by the cadaver's sporulation. All larvae exposed to the fungi died, usually

Table 3 Pathogenicity assay of all the isolates collected from Central India

Name of fungus	Name of insect	Range of mortality (%)	No. of isolates*
<i>Beauveria bassiana</i>	<i>Hyblaea puera</i>	65–95	95
		40–60	51
		10–30	29
<i>Nomuraea rileyi</i>	<i>Diachrysia orichalcea</i>	60–95	25
		35–55	8
<i>Paecilomyces fumosoroseus</i>	<i>Hyblaea puera</i>	45–65	9
		10–20	6
<i>Paecilomyces farinosus</i>	<i>Hyblaea puera</i>	30–60	15
<i>Metarhizium anisopliae</i>	<i>Hyblaea puera</i>	40–70	5
<i>Lecanicillium lecanii</i>	<i>Hyblaea puera</i>	30–45	2
<i>Isaria felina</i>	<i>Hyblaea puera</i>	20	1

*Number of test isolates fall in one category of the percentage of mortality caused against the test insect species.

within 4 days. On the other hand, these indigenous isolates from Central India varied in their virulence, although their inoculation experiments were conducted at a similar conidial concentration. The pathogenicity assays showed that some isolates of *B. bassiana*, *P. farinosus* and *P. fumosoroseus* either recovered from *H. puera* or other insect hosts showed higher potential for killing the teak defoliator, *H. puera*, the most common and serious teak pest in India, whereas the isolates of *N. rileyi* also showed higher potential for killing soybean pest, *D. orichalcea*.

No mycosis was found on the insects of the control plot, and there was apparently no fungal contamination among the experimental insects. No larva molted within a few days after inoculation, and the inoculated fungi were thought to have completed penetration into larval hemocoel, although some molted many days after the inoculation. There is an indication that these isolates may have potential for reduction of pest species. These experiments were carried out in the laboratory; and further field experiments for examining the pathogenicity of these potential isolates in a tropical environment is yet to be carried out in Jabalpur.

Discussion

In Central India, there are no biological control agents for use against insect pests. Current research efforts are focused on selecting indigenous entomopathogens, which are highly virulent to arthropod pests, for developing

efficient and environmentally sound bioinsecticides. Clearly, entomopathogenic fungi are important natural enemies of various arthropods in forests and agricultural fields of Central India among which *B. bassiana* was the predominant fungus in these surveys. The surveys described in this study provided an important source of basic scientific knowledge and simultaneously the collection of a large number of samples for further investigation. Understanding the fungal flora is a prerequisite for many basic mycological and applied scientific studies, and for the management of natural resources. Similarly effective research needs adequate and reliable sources of properly preserved cultures [24].

The occurrence of the fungi collected during these surveys was probably influenced by many factors, such as habitat environment, macro and microclimate, availability of hosts [25]. The richness of fungi in our collection is low compared to that recorded for tropical habitats [26] and the estimated global diversity that exceeds 700 species [27], but it is similar to that recorded for other temperate regions [28]. It should be noted, however, that this study gave no indication as to the extent of intraspecific diversity, which could be considerable in entomopathogenic fungi [29].

The prevalence of *B. bassiana* was markedly higher in forest habitat than the agriculture. Other species except *N. rileyi*, which is restricted to agricultural fields, were found only in the forest habitats. The lower frequency of entomopathogenic fungal species in intensely cultivated areas than in forest areas have been reported from several regions. However, individual fungal species often exhibit differing responses. *M. anisopliae* is common in both natural and cultivated habitat, and *P. fumosoroseus* occur only in natural habitats or in relatively undisturbed cultivated habitats. *B. bassiana* and *P. farinosus* were found to be very sensitive to the disturbance effects of cultivation and thus were restricted to natural habitats [30].

In the present study, *Paecilomyces* species were isolated from larvae of *H. puera*, *E. machaeralis*. Already there have been records of isolating *Paecilomyces* species from other species of lepidopteran [31] but there has been no record of this genus from stick insect, spider and giant beetle. Although these *Paecilomyces* have weak virulence, but were found to be infectious to *H. puera* when inoculated. Thus, it would be possible to find isolates or species of *Paecilomyces* that could have higher pathogenicity against important insect pests. No record of *I. felina* in association with an arthropod host was reported earlier. Isolation of *I. felina* from an insect host (beetle) is therefore the first Central Indian record in this regard.

Conclusion

The systematic, biodiversity and conservation biology programs within this and most other departments of mycology at all the Indian National Institutions are generally perceived as being at the basic end of the basic-applied continuum. Systematic, in particular, is concerned with fundamental questions dealing with taxonomic diversity, patterns of evolution, and natural history. The fact that systematic mycology forms a substantial amount of the foundation of all other mycological research, whether basic or applied, requires that it should be included in the overall research program of any healthy department of mycology [32].

The diversity and distribution of entomopathogenic fungi occurring in Central India could play an important role in regulating the forest and agricultural pest populations. Additional research is needed to clarify phylogenetic relationships among all the species of indigenous entomopathogenic fungi collected during these surveys. Morphological characters are limited in their ability to predict the species therefore; PCR-based molecular analysis [33, 34] will be valuable in future population studies, which will further help in developing a long-term management and conservation program required to protect these species. The selection of fungal entomopathogens for further development as commercial mycoinsecticide is possibly the most critical step in this process.

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