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Occurrence and Distribution of *Cryptococcus* Species in Environmental Sources from Lower Assam Belt of India

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Abstract

Cryptococcus **is a non-motile, gram positive, non-fermenting Basidiomycetous encapsulated yeast like fungus that causes respiratory, neurological and other systemic diseases in both humans and animals. Present study delineates the possible distribution of *Cryptococcus* species in pigeon droppings, excreta of other avian species, eucalyptus tree and contaminated soil specimens collected from different geographical co-ordinates of six geographical regions of the lower Brahmaputra Valley of Assam, India. The fungi were isolated through conventional methods of Sabouraud Dextrose Agar (SDA) and Bird Seed Agar (BSA) media and identified through negative staining of capsule as well as performing classical bio-chemical tests. Identity of the isolates was further confirmed through sequence analysis of ITS-1 and ITS-4 region of the 18S rDNA. Two pathogenic species of *Cryptococcus* were isolated from 67 (15.40%) of the 435 specimens. Of these positive isolates 41 (9.43%) belonged to *Naganishia albida* (*Cryptococcus albidus*) while 26 (5.98%) represented *Papiliotrema laurentii* (*Cryptococcus laurentii*). Both the species were recovered from 58 (18.35%) dry and 9 (7.56%) moist specimens. The percentage of prevalence of *Naganishia albida* in dry and moist specimens were 35 (11.07%) and 6 (5.04%) respectively. Contrary to this, the percentage of prevalence of *Papiliotrema laurentii* in dry and moist were 23 (7.28%) and 3 (2.52%) respectively. The findings indicate that *Cryptococcus* species have established a better ecological sustenance in dry specimens than moist. The findings of the investigation demonstrated that the prevalence of *Cryptococcus albidus* in attics, dovecotes / houses of pigeon fanciers, contaminated soil, eucalyptus tree and droppings of other birds were 11(12.36%) out of 89, 23(14.11%) of 163,2(3.23%) of 62,4(7.84%) of 51 and only 1(1.43%) out of 7 specimens respectively. The recovery of *Papiliotrema laurentii* in the above specimens were 3(3.37%), 20(12.27%), 1(1.61%), 1(1.96%) and 1(1.42%) respectively. The findings revealed that the prevalence of *Naganishia albida* is more than that of *Papiliotrema laurentii* in natural substrates. The notorious pathogenic fungi, *Cryptococcus neoformans* could not be isolated, indicative of the fact that the region selected for the study is not environmentally favorable for growth and sustenance of the species. Findings of the study clearly demonstrate the ecological and epidemiological significance of the non-neoformans species of the genus *cryptococcus* that needs further comprehensive studies to access the prevalence of the genus from public health point of view.**

Keyword: *Cryptococcus*, pigeon droppings, natural substrates, ecological relationship, lower Brahmaputra valley, prevalence

INTRODUCTION

Cryptococcus is non-motile, gram positive, non-fermenting *Basidiomycota* from the order *Tremellale* under the family of *Tremellaceae* of capsulated yeast like fungus [1]. Littman (1959) reported the first case of cryptococcosis in man directly attributed to pigeon excreta [2]. Outbreaks of cryptococcosis are reported in human, animals and avian species whilst presence of the causative organism has been reported in reptiles, fruits and vegetables [3]. Of more than 70 species of the genus *Cryptococcus*, a majority thrive in the environment and only few of them are medically important disease causing pathogenic agents [4]. Amongst all the species, *Cryptococcus neoformans* is regarded as the major human and animal pathogen, while *Cryptococcus albidus* and *Cryptococcus laurentii* are occasionally known to cause moderate to severe diseases especially in immuno-compromised patients [5].

Cryptococcus species are commonly found in dropping of pigeons that apparently harbors the organism in normal commensal form [6]. In addition, the digestive tracts of parrots and canaries also harbour the fungus [7]. Pigeon droppings, are available in most unlikely places such as roofs and ventilations of abandoned buildings, cornices, leaves and branches of trees that serve as ecological niche for adaptation, dispersion of *Cryptococcus* replication and transmission. Other environmental sources such as fruits and vegetables retain the fungi as saprophyte that may cause infection in man and animals either by inhalation of spores of the organism or through subcutaneous inoculation [3,8–10].

Excreta of pigeon as saprobic reservoir of *Cryptococcus* species have been frequently recovered from various countries of the world [3,11,12]. Isolation of *Cryptococcus* from many tree

species in America [13] Brazil [14], India [15], Iran [16] have been reported.

Among the systemic and opportunistic mycoses, cryptococcosis continues to exacerbate severe health risks, especially in high risk groups and immuno-compromised patients. It has been estimated that the global prevalence of cryptococcosis among AIDS patients stands at 2.33% and 6.8% both worldwide and within India respectively [3]. However, incidence of cryptococcosis in recent years is alarmingly increasing at a global scale amounting to one million infections and approximately 6,25,000 deaths annually, rendering the disease to be considered as an most important fungal diseases [17].

Cryptococcosis is a highly infectious and enigmatic mycotic disease that affect a variety of animals too [18,19]. The disease occurs in acute, sub-acute or in chronic forms and have global significance [20]. The fungi causes respiratory as well as neurological problems and is often sporadic in nature [19,21,22]. Incidentally, the exact epidemiological data on the incidence and prevalence of the disease is not readily available as the disease is not a notifiable one although several reports of considerable morbidity and mortality in human as well as in animals are well documented [21].

The first report of Cryptococcosis in India was documented in 1952 and subsequently many other investigators reported its occurrence from time to time in various parts of the country [23]. Although numerous studies on the zoonotic importance and epidemiology of *Cryptococcus neoformans* have been reported, yet there is lack of proper information as literatures are scanty on the non-neoformans species like *Cryptococcus albidus, Cryptococcus laurentii* or *Cryptococcus uniguttulatus* [7]. In recent years, a burst of increase in opportunistic infection by these non-neoformans pathogenic yeasts have been observed [24]. Environmental sources including canopy leftovers from some trees are regarded as the main sources of *Cryptococcus albidus, Cryptococcus laurentii,* and *Cryptococcus uniguttulatus* that lead to infection in human and animals [16,25,26].

Epidemiology of non-neoformans *Cryptococcus albidus*, *Cryptococcus laurentii, Cryptococcus uniguttulatus, Cryptococcus luteolus* and some other species of the genus are highly relevant as these species often turn out to be pathogenic and thereby increases the risk of infection. A case of encephalitis in an HIV patient due to *Cryptococcus albidus* was reported in China [27] while fungal keratitis due to the same species was reported by Huang *et.al*, (2015) [28]. Earlier, pulmonary Cryptococcosis due to *Cryptococcus laurentii* in diabetis and patients suffering from ganglio-neuroblastoma were reported by Averbuch *et.al*, (2002) [29] and Shankar *et.al*, (2006) [30] respectively. Reports are also available on Cryptococcal meningitis, Cryptococcal myelitis and multiple skin lesions in HIV patients infected with *Cryptococcus neoforma*ns var. *grubii* from Assam having a history of occupational exposure to pigeons and chicken [31].

Such findings are of greater significance to places like Assam where domestication of pigeons, fowls and various birds of the finch family are common among the inhabitants at a large scale both as household pets and for meat. A proper surveillance of the sites of occurrence of the fungi as well as characterization of its diverse forms for creating a database is a primary need since the practice of rearing pigeons is non-scientific, traditional and sporadic reports of pulmonary diseases (including cryptococcal meningitis) have been recorded from time to time amongst the common mass. There is a remarkable gap of information on this aspect in the entire northeastern part of India, including Assam. This is a preliminary attempt for identifying the hot spots of *Cyptococcus* occurrence in environmental samples representing the lower Brahmaputra valley of Assam and characterizing the isolates for their proper identification to generate a baseline data. It is envisaged that the study will generate information for future planned research activities on the opportunistic systemic mycotic disease causing fungi.

MATERIALS AND METHOD

In the present investigation, a total of 435 samples of pigeon droppings and droppings of other avian species, barks and leaf litter of natural stands of eucalyptus trees as well as environmental soil from different sources from five different distinct regions of the lower Brahmaputra

Valley of Assam were collected. These included the districts of Bongaigaon, Dhubri –(both north bank extending up to West Bengal border and south bank extending upto the border of the state of Meghalaya along the river Brahmaputra), the district of Kokrajhar BTAD region, the district of Goalpara extending up to the border with Meghalaya, the district of greater Kamrup (including both urban and rural extensions along the north bank of the river Brahmaputra) and finally the district of Nalbari of western lower Assam. Samples collected were transported to the laboratory in ice cooled boxes packed in polyethylene bags. However, cloacal samples of pigeon were collected using sterile cotton swab in a transport media (Eurotubo^{r}, Rubi Barcelona, Spain). After collection, all samples were either processed on same day or stored at 4^{0}C until being processed for isolating *Cryptococcus* species. In the meantime, basic environmental characteristics of the collection areas were noted for understanding the ecological and epidemiological relevance of the organism collected from different sources (Table.1, Fig.1, Fig.2 and Fig.3). The marked samples were processed as per standard methods mentioned elsewhere with slight modifications [14,32].

Isolation and Identification

Processing of samples was done in the Department of Biotechnology, Gauhati University. Two grams (2g) of the samples was diluted in 20 ml of sterile physiological saline and the mixture was stirred for five minutes in a vortex apparatus and allowed to stand for 15-20 minutes for

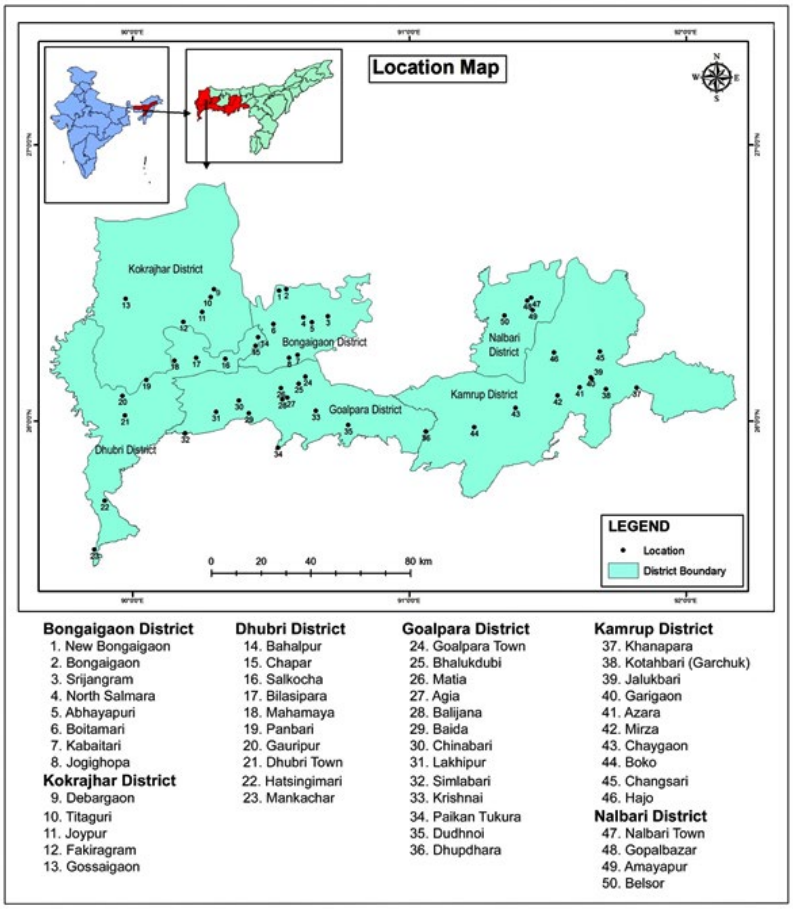

Fig. 1. Representation of sample collection areas developed from GPS coordinates and using GIS software prepared by Aaranyak, Guwahati, Assam

decantation. After this, 1 ml of the supernatant was inoculated onto 9 ml of sterile physiological saline supplemented with chloramphenicol (0.05mg/ml) and then incubated at 37^{0}C for an hour. An aliquot of 0.1ml of each supernatant of the processed samples were aspirated and streaked onto duplicate plates of Sabouraud Dextrose Agar (SDA) and a Bird Seed Agar (BSA) supplemented with chloramphenicol (0.05mg/ml).

Cultures with SDA media were incubated at 37^{0}C while that of Bird Seed Agar (BSA) were incubated at 25^{0}C for 15 days and monitored daily from 2nd day onward for observing growth and presence of colony and to evaluate colony morphology. Brown pigmented colonies were identified as *Cryptococcus neoformans* while colonies showing cream colouration with smooth and mucoid appearance were identified as non-neoformans species coglomerate. Results evaluated on the basis of morphological data were expressed as the average nos. of viable yeast cells per gram of sample (CFU/g). For comparisons, *Cryptococcus laurentii* MTCC 2898 (procured from CSIR Institute of Microbial technology, Chandigarh – 1600361, India) was used as control. Identified isolates were sub cultured, purified and further subjected to an array of morphological, cultural and bio-chemical tests such as carbohydrates, nitrite reduction, urease production and phenol oxidation [11,15,16,20,24].

Capsule Identification

For capsule identification of the probable *Cryptococccus* spp., a drop of inoculum from newly inoculated isolates grown on SDA was added onto India ink stain on a sterile glass slide with a cover slip and observed under a bright

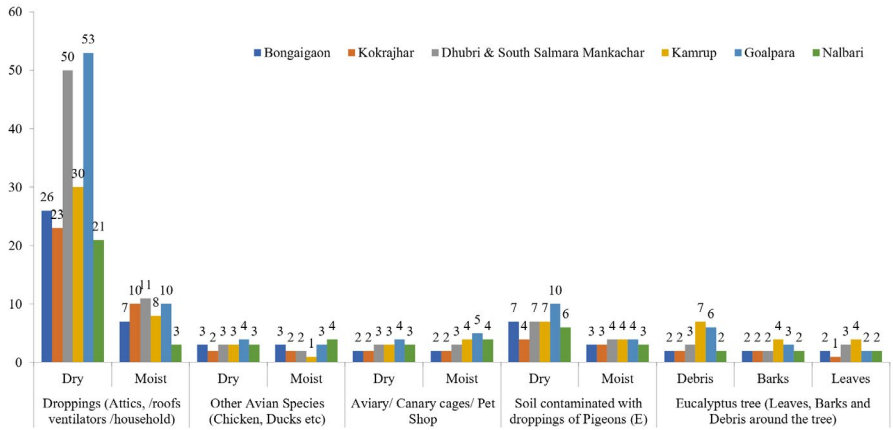

Fig. 2. Frequency distribution of samples as per their sources and types

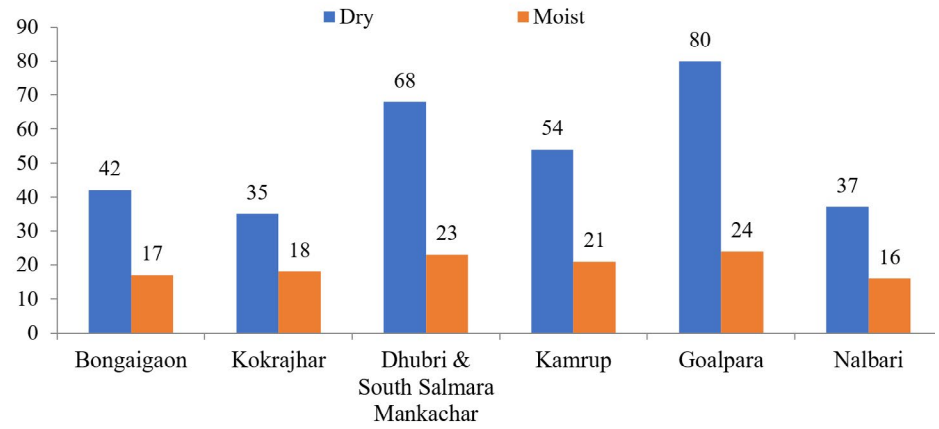

Fig. 3. Distribution of samples collected from different regions and their type

field microscope (Olympus CX33) at 100 and 400 times magnification (Kwon-Chung and Bennett, 1992) and also phase contrast microscopy (Leica DM750) under 1000 fold magnification. Presence of distinct wide round to oval gelatinous capsule, with or without hyphae was considered as positive observation [33–36].

Molecular Characterization

DNA extraction

For DNA extraction, isolates from pure cultures were inoculated onto 1.5ml Eppendorf tubes containing 0.5 ml of Sabouraud Dextrose broth supplemented with chloramphenicol and incubated overnight in an orbital shaker at 150 rpm and 30°C. After 24 hours, the fungal suspensions with predetermined concentrations were centrifuged at 5,000 rpm for 10 minutes, and the pellet was frozen at -20°C for 1 hour with further incubation at 65°C for 1 h in 0.5 ml of extraction buffer containing 50 mM Tris-HCl, 50 mM EDTA, 3% sodium dodecyl sulfate and 1% 2-mercaptoethanol (Ferrer et al., 2001). The lysate was finally extracted with phenol-chloroform-isoamyl alcohol (v/v) in the ratio 25:24:1. To this, 65µl of 3M sodium acetate and 75µl of 1M NaCl were added and the resulting volume was incubated at 4°C for 30 minutes. DNA was recovered by isopropanol precipitation and washed with 70% (v/v) ethanol. Concentration of DNA was measured at 260 nm in a UV-VIS Spectrophotometer (Shimadzu) and stored at -20°C until further use [37–39].

PCR amplification

For characterization of *Cryptococcus* species, isolated DNA was amplified in a gradient PCR (Eppendorf Nexus Gradient). The primers

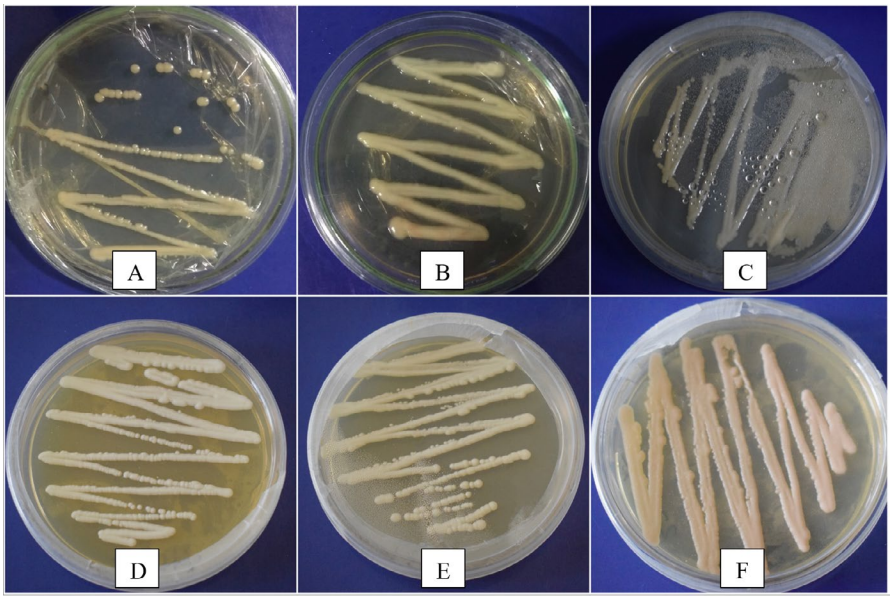

Fig. 4. ((A) Reference strain) *Papiliotrema laurentii* (MTCC 2898), (B, C) *Papiliotrema laurentii*, (D, E, F) *Naganishia albida*.

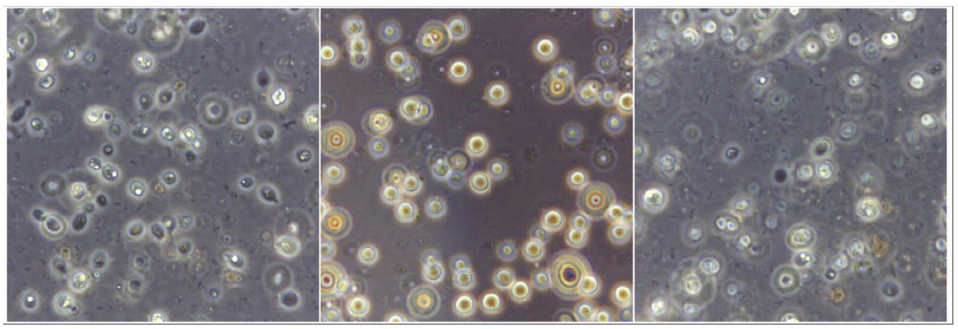

Fig. 5. Phase contrast microscopy of reference sample - *Papiliotrema laurentii* (MTCC 2898), under 100X magnification

Table 1. Frequency distribution of samples as per their sources and types

Breakup of samples from different sources and geographical regions																	
					Sources of Samples												
Sl No	Sample Code	Area/ Region/ District	Geographical Location/ Place of Sampling	Season/ Month/ Year	Pigeons Droppings (A)				Other Avian Species (Chicken, Ducks etc.) (C)		Aviary/ Canary cages/ Pet Shop (D)		Soil contaminated with droppings of Pigeons (E)		Eucalyptus tree (Leaves, Barks and Debris around the tree) (F)			Total Sample Dry & Moist
					Attics, / roofs ventilators of old buildings (A)		Pigeon fanciers house holds (Cloacal/ Fresh) (B)											
					Dry	Moist	Dry	Moist	Dry	Moist	Dry	Moist	Dry	Moist	Debris	Barks	Leaves	
1	BNG	Bongaigaon	Jogighopa, North Salmara, Abhayapuri, Baitamari, Bongaigaon Town, Kabaitari, Chalantapara	July, August, September, October/2018 and March/2017	8	2	18	5	3	3	2	2	7	3	2	2	2	Dry-42 Moist-17 Total = 59
2	KJR	Kokrajhar	Jaipur, KokrajharTown, Titaguri, Devorgaon	July, August, September/2018 and Feb, March/2017	8	4	15	6	2	2	2	2	4	3	2	2	1	Dry-35 Moist-18 Total = 53
3	DHB	Dhubri and South Salmara Mankachar	Bahalpur, Chapar Bilasipara, Gauripur, Dhubri Town, Hatsingimari (Meghalaya Border), Mahamaya temple area	June, July, September, October/2018 and Feb, March/2017	21	3	29	8	3	2	3	3	7	4	3	2	3	Dry-68 Moist-23 Total = 91
4	KRP	Kamrup Rural and Urban	Mirza, Kotahbari, Gorchuk, Azara, Hajo, Jalukbari, Chaygaon, Boko	June,July, August, September, October/2018 and February/2017	11	4	19	4	3	1	3	4	7	4	7	4	4	Dry-54 Moist-21 Total = 75
5	GLP	Goalpara	Lakhipur, Simlabari Tikrikilla, Meghalaya border, Agia, Krishnai, Dudhnoi,Matia, Dhupdhara and Goalpara	March/2017 and June, July, August, Septmber/2018	19	3	34	7	4	3	4	5	10	4	6	3	2	Dry-80 Moist-24 Total = 104
6	NLB	Nalbari	Nalbari town, Gopal Bazar, Belsor, Chamata, Amayapur.	Sep, Oct/2018 and Feb, March, April/2017	5	1	16	2	3	4	3	4	6	3	2	2	2	Dry-37 Moist-16 Total = 53
Total					72	17	131	32	18	15	17	20	41	21	22	15	14	Total Dry-316 Moist-119 Total = 435

used for amplification included the D1/D2 regions targeting ITS1 and ITS4 with expected fragment length of 600 bp. Details of the primers are given () in Table 2. The program for amplification was set for 1 cycle of initial denaturation for 30 minutes at 94^{0}C followed by 35 cycles of denaturation for 1 minute at 94^{0}C, primer annealing for 1 minute at 55^{0}C, chain extension for 1 minute at 72^{0}C and a final extension for 7 minutes at 72^{0}C respectively. The PCR products after amplification were resolved in a 1.5 % agarose gel subjected to electrophoresis and was visualized under UV gel documentation system (UVitec Cambridge, Genei). The amplicons were later stored at -20^{0}C for further analysis [40–43].

Sequencing

Sequencing of PCR products were done at Xcelris Labs Limited, India through outsourcing. Chromatogram files obtained were analyzed for nucleotide-BLAST on NCBI portal for identification of the species. Phylogram and dendrogram was prepared using PhyML (Phylogenetic Maximum Likelihood) (http://www.atgc-montpellier.fr/phyml/).

Statistical analysis

A very brief descriptive analysis was carried out to interpret the data as they were mostly qualitative attributes. However, positive results obtained from different sources were compared through k-proportion test through Monte Carlo / Marascuilo methods to assess their homogeneity across sources with the help of XLSTAT software [44–46].

RESULTS

In the present study, a total of 67 *Cryptococcus* spp were recovered from 435 samples with a prevalence of 15.40% (Table 3). Out of 67 positive isolates, 41 (9.43%) were identified as *Cryptococcus albidus* (*Naganishia albida*) Table 3, Fig. 4, Plate-D, E, F while 26 (5.98%) were identified as *Cryptococcus laurentii* (*Papiliotrema laurentii*). Details of the identified (Table. 3, Fig. 4, Plate-B, C). Cultural and biochemical characteristics of *Naganishia albida* and *Papiliotrema laurentii* were almost similar except in the utilization of potassium nitrate which was negative in case of *Papiliotrema laurentii*. Cultural characteristics of *Cryptococcus laurentii* (Fig. 4, Plate B, C) in SDA media were comparable with the reference strain MTCC 2898 (Fig. 4, Plate A). Based on the type of sample (dry and moist), *Naganishia albida* (*Cryptococcus albidus*) and *Papiliotrema laurentii* (*Cryptococcus laurentii)* could be isolated from 58 (18.35%) of dry and 9 (7.56%) of moist specimens collected from different geographical niches / co-ordinates. **(**Table 3, Fig. 7).

Table 2. Sequence of primers used for PCR amplification

Primer	Sequence
ITS1	3'- TCC GTA GGT GAA CCT GCG G -5'
ITS4	5'- TCC TCC GTC TAT TGA TAT GC -3'

During the investigation of 57 (22.61%) *Cryptococcus* species isolated from pigeon droppings from sites A & B, 34 (13.49%) were identified to be *Cryptococcus albidus* while 23 (9.13%) represented *Cryptococcus laurentii*. The highest recovery percentage of *Cryptococcus* (57) was observed in pigeon droppings and the least (2) was from avian species other than pigeon. In terms of relative isolation of positive cultures of *Cryptococcus*, 14 (15.73%) were from attics / ventilations / old and abandoned buildings (Site A), 43 (26.38%) were from houses of pigeon fanciers / dovecots (Site B), 3(4.84%) were from contaminated environment / soil (Site C), 5 (9.80%) were from the debris / barks / leaves of eucalyptus trees (Site D) while 2 (2.86%) were from other avian sources like chicken, duck, parrot etc (Site E). The results are presented in Table 3.

Relative and absolute percentage of isolates of *Cryptococcus* (*Naganishia albida* and *Papiliotrema laurentii*) from each collection site was also analyzed. Prevalence rate of isolates from Site A (3.22%), Site B (9.89%), Site C (0.69%), Site D (1.15%) and Site E (0.46%) was recorded (Table 4). Results interpret that there exist a significant difference at 0.05% among the variants of different proportions obtained from various samples during the study. Values having the superscript across different rows do not differ significantly.

The overall prevalence of *Naganishia albida*, across all dry and most environmental samples was 9.43% (41 of 435). However, the prevalence of the species was 35 (11.07%) in dried specimens in comparison to 6 (5.04%) from moist specimens. Details of prevalence of *Naganishia*

Table 3. Frequency distribution of positive samples of Cryptococcus species (pooled sample)

Species of Cryptococcus	(A)			Pigeon droppings with code no. (B)			(A+B)			Pigeon droppings contaminated with soil(C)			From Eucalyptus Trees (D)				Droppings from other avian species and aviary/Canary cages/ Pet shops nos and %			Total positive samples from all sources (F)		
	Attics/Ventilators /roofs of private and public places/ old and abandoned buildings (nos. and %)			Houses of Pigeon fanciers /dovecotes (nos and %)			Pigeons dropping Attics & dovecotes (nos and %)			nos and %			nos and %				nos and %			nos and %		
	Dry	Moist	Total	Dry	Cloacal/ Moist	Total	Dry	Moist	Total	Dry	Moist	Total	Debris (dry)	Barks (dry)	Leaves (moist)	Total	Dry	Moist	Total	Dry	Moist	Total
Total nos of samples	72	17	89	131	32	163	203	49	252	41	21	62	22	15	14	51	35	35	70	316	119	435
Naganishia albida (positive & %)	10 (13.89)	1 (5.88)	11 (12.36)	20 (15.27)	3 (9.37)	23 (14.11)	30 (14.78)	4 (8.16)	34 (13.49)	2 (4.88)	0 (0.00)	2 (3.22)	3 (13.64)	0 (0.00)	1 (7.14)	4 (7.84)	0 (0.00)	1 (2.85)	1 (1.42)	35 (11.07)	6 (5.04)	41 (9.43)
Papiliotrema laurentii(positive & %)	3 (4.17)	0 (.00)	3 (3.37)	18 (13.74)	2 (6.25)	20 (12.27)	21 (10.34)	2 (8.08)	23 (9.13)	1 (2.44)	0 (0.00)	1 (1.61)	0 (0.00)	1 (6.67)	0 (0.00)	1 (1.96)	0 (0.00)	1 (2.85)	1 (1.42)	23 (7.28)	3 (2.52)	26 (5.98)
Naganishia albida & Papiliotrema laurentii (ppositive& %)	13 (18.05)	1 (5.88)	14 (15.73)	38 (29.07)	5 (15.62)	43 (26.38)	51 (25.12)	6 (12.24)	57 (22.61)	3 (7.32)	0 (0.00)	3 (4.84)	3 (13.64)	1 (6.67)	1 (7.14)	5 (9.80)	0 (0.00)	2 (5.71)	2 (2.85)	58 (18.35)	9 (7.56)	67 (15.40)

albida is presented in Table 5 whilst morphological features of the species is presented in the Fig 4 (D, E, F) as well as Fig. 6B, and Fig. 8 respectively. Results interpret a significant difference at 0.05 % level among the different proportions obtained from different sources during the study. On the contrary, the overall prevalence of *Papiliotrema laurentii* in all dried and moist environmental samples were 5.98% (26 of 435), 7.28% (23 of 316) and 2.52% (3 of 119) respectively from different sites. Details of the prevalence of the species is presented in Table. 6, Fig. 6(A) and Fig. 9 alongwith the comparable datasheet of reference strain (*Cryptococcus laurentii* MTCC 2898, Fig. 5). Result correlate a significant difference at 0.05 % level among the representative proportions obtained from different sources during the study. Details of genetic identity of the isolated strains of *Naganishia albida* and *Papiliotrema laurentii* including sequenced data of the rDNA ITS region and their accession numbers obtained from NCBI after submission of the sequences are presented in Table. 7 and Fig. 10 respectively.

Comparisons pertaining to the prevalence of *Naganishia albida and Papiliotrema laurentii* across the studied geographical locations depict an overall 11 (18.64%), 16 (17.58%), 9 (16.98%), 16 (15.38%). 10 (13.33%) and 5 (9.43%) numbers of positive isolates representing the districts of Bongaigaon, Dhubri, Kokrajhar, Goalpara, Kamrup and Nalbari (Table. 8; Fig. 11). We could not establish any significant difference in the results observed across different districts. Moreover, the prevalence of pathogenic *Cryptococcus neoformans* could not be observed from any of the tested samples indicating absence of the species in the specimens collected which may be due to unfavorable habitat niche for the species along the lower Brahmaputra valley of Assam resulting in complete absence of the most feared representative form of the family of *Cryptococcus*.

DISCUSSION

Cryptococcosis is a major public health concern in India. Epidemiology and pathogenicity of this enigmatic myco-zoonosis in man and animals have been well studied [18–21]. Isolation and identification of *Cryptococcus* from the environment demonstrates the importance of bio-diversity and environmental niche of the pathogenic

Table 4. Isolates of *Cryptococcus* Species Naganishia albida (cryptococcus albidus) and Papiliotrema laurentii(cryptococcus laurentii) from different sources of samples

Sl No	Collection site	Environmental niches	n	Dry Positive	Relative & absolute isolations/ isolations (No & %)	n	Moist Positive	Relative & absolute isolations/ proportions (No & %)	N	Total Positive	Relative & absolute isolations/ proportions (No & %)
1	Attics / ventillations of old abandoned building (A)	Pigeon droppings (*Columba livia*)	72	13	18.05 (4.11)	17	1	5.88 (0.84)	89	14	15.73 (3.22)
2	Houses of Pigeon fancies / dovecotes (B)	Pigeon droppings (*Columba livia*)	131	38	29.00 (12.02)	32	5	15.62 (4.20)	163	43	26.38 (9.89)
3	Environmental samples/ soil (C)	Contaminated with pigeon droppings	41	3	7.32 (0.95)	21	0	0	62	3	4.84 (0.69)
4	Eucalyptus tree (D)	Debris, barks (dry) and leaves (moist)	37	4	10.81 (1.27)	14	1	7.14 (0.84)	51	5	9.80 (1.15)
5	Other birds (E)	Parrots, chicken, ducks etc.	35	0	0.00	35	2	5.71 (1.66)	70	2	2.86 (0.46)
6	Total samples (F)		316	58	18.35	119	9	7.56	435	67	15.40

Figures in parenthesis indicate percent with respect to total sample size (N), n = number of samples for both dry and moist samples.
* P = 0.001 (<0.05)

Table 5. Isolates of Naganishia albida (Croptococcus albidus) from different sources of samples

Sl No	Collection site / Sources	Environmental niches	Collected samples/ Positive isolates						Absolute isolations/ relative isolations (No & %)		
			Dry			Moist			Total		
1	Attics / ventillations of old / abandoned / building (A)	Pigeons' droppings (C livia)	n	Positive	Relative & absolute isolations/ isolations (No & %)	n	Positive	Relative & absolute isolations/ isolations (No & %)	N	Positive	Relative & absolute isolations/ isolations (No & %)
			72	10	13.89 / 3.16	17	1	5.88/0.84	89	11[ab]	12.36 / 2.52
2	Houses of Pigeons' fancies / dovecotes (B)	Pigeons' droppings (C livia)	131	20	15.27 / 6.33	32	3	9.37/2.52	163	23[b]	14.11 / 5.29
3	Environmental samples/ soil (C)	Contaminated with pigeons' droppings	41	2	4.88 / 0.63	21	0	0.00	62	2[a]	3.23 / 0.46
4	Eucalyptus tree (D)	Debris and barks (dry) and leaves (moist)	37	3	8.10 / 2.59	14	1	7.14/0.84	51	4[ab]	7.84 / 0.22
5	Other birds (E)	Parrots, chicken, ducks etc.	35	0	0.00	35	1	2.85/0.22	70	1[a]	1.43 / 0.84
6	Samples Total (F)		316	35	11.07	119	6	5.04	435	41	9.43

Figures in parenthesis indicate percent with respect to total sample size (N), 'n' indicate number of samples
(Values having the superscript across different rows do not differ significantly)

* P = 0.008 (<0.05)

species and strains of this basidiomycota yeast type fungus because such species usually increase the risk of infection in susceptible population exposed to hypersensitive reactions. Besides, vulnerability of infection among the immuno-compromised hosts other than the infectious state

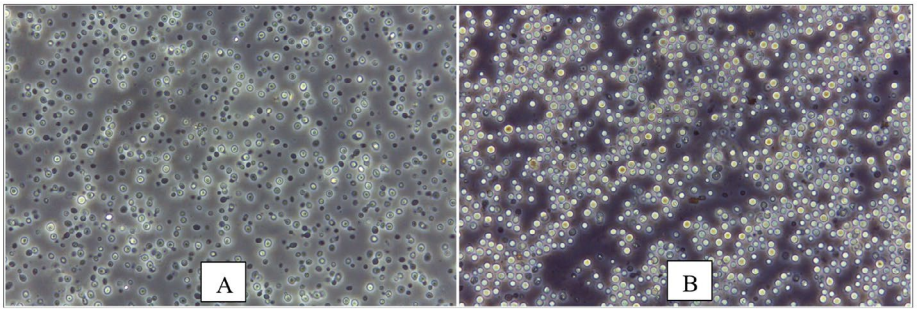

Fig. 6. Phase contrast microscopy of (A) *Papiliotrema laurentii* (B) *Naganishia albida* under 40x magnification.

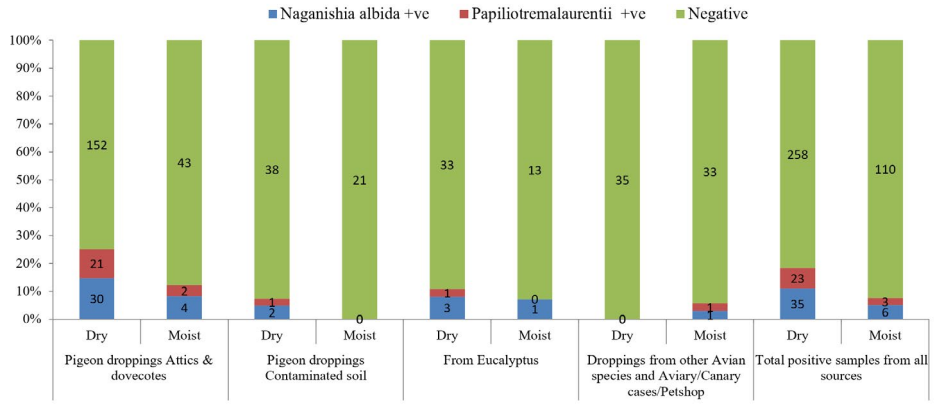

Fig. 7. Distribution of positive samples of *Cryptococcus* species isolated from samples and specimens

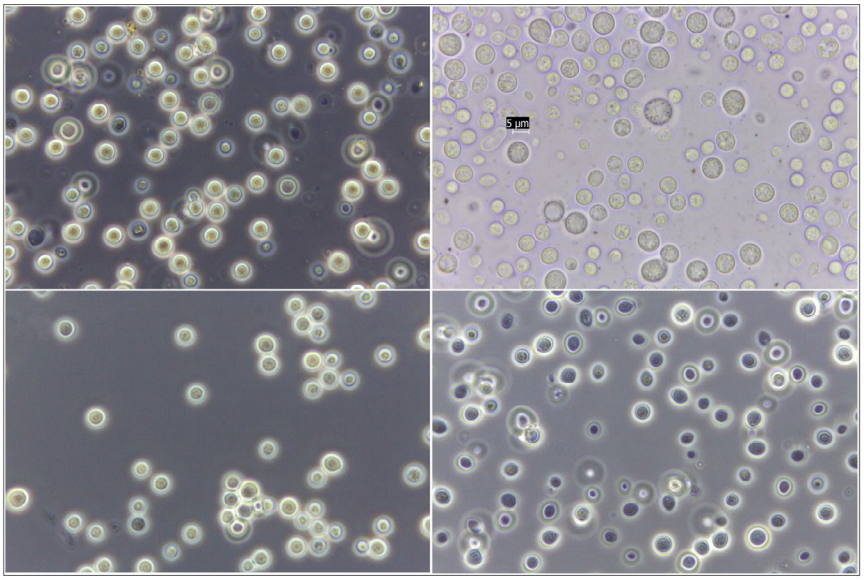

Fig. 8. Phase contrast microscopy of *Nagashia albida* under 100x magnification

Table 6. Isolates of *Papiliotrema laurentii* (*Cryptococcus laurentii*) from different sources of samples

No.	Collection site	Environmental niches	Collected samples/ Positive isolates						Absolute isolations/ relative isolations (No & %)		
			Dry			Moist			Total		
			n	Positive	Relative & absolute isolations/ isolations (No & %)	n	Positive	Relative & absolute isolations/ isolations (No & %)	N	Positive	Relative & absolute isolations/ isolations (No & %)
1	Attics / ventillations of old / abandoned/ building (A)	Pigeons' droppings (*Columba livia*)	72	3	4.17/0.95	17	0	0.00	89	3	3.37/0.69
2	Houses of Pigeons' fancies /dovecotes (B)	Pigeons' droppings (*Columba livia*)	131	18	13.74 / 5.70	32	2	6.25 (0.69)	163	20	12.27 / 4.60
3	Environmental samples/ soil (C)	Contaminated with pigeons' droppings	41	1	2.44 / 0.32	21	0	0.00	62	1	1.61 / 0.23
4	Eucalyptus tree (D)	Debris and barks (dry) and leaves (moist)	37	1	2.70/0.32	14	0	0.00	51	1	1.96 / 0.23
5	Other birds (E)	Parrots, chicken, ducks etc.	35	0	0.00	35	1	2.85 (0.84)	70	1	1.42 / 0.23
6	Samples Total (F)		316	23	7.28	119	3	2.52	435	26	5.98

Figures in parenthesis indicate percent with respect to total sample size (N), 'n' indicate number of sample
(Values having the superscript across different rows do not differ significant)
* P = 0.001 (<0.05)

of different biovars of this pathogen, includes both pathogenicity and antifungal resistance which have tremendous implications. Ecological relationship of *Cryptococcus* was not known until Emmons (1955)[10] found the fungus in droppings of pigeon and soil colonized by the genera. Later, his observation was substantiated to be true by many researchers [2,3,17,20,24].

Although many studies have been conducted on the epidemiology and pathogenicity of *Cryptococcus neoformans*, yet there is scarcity of current information on the prevalence, epidemiology and pathogenicity of the non-neoforman species. The present study defines the incidence of non-neoforman species of the genus *Cryptococcus* inherent in pigeon droppings, ecological niche of eucalyptus trees and in other microfloral sources of the environment at a specific geographical region along the lower Brahmaputra valley of Assam.

Our observation confirmed the presence of two non-neoforman species of the genus *Cryptococcus*, viz., *Naganishia albida* and *Papiliotrema laurentii*. To our knowledge, this is the first report on the isolation of these two species from pigeon droppings and other environmental sources of the northeastern region of India and this may be considered as an established fact that the environmental sources of the studied areas serve as ideal saprobic reservoirs of these two opportunistic pathogens. Considering the importance of the species at the backdrop

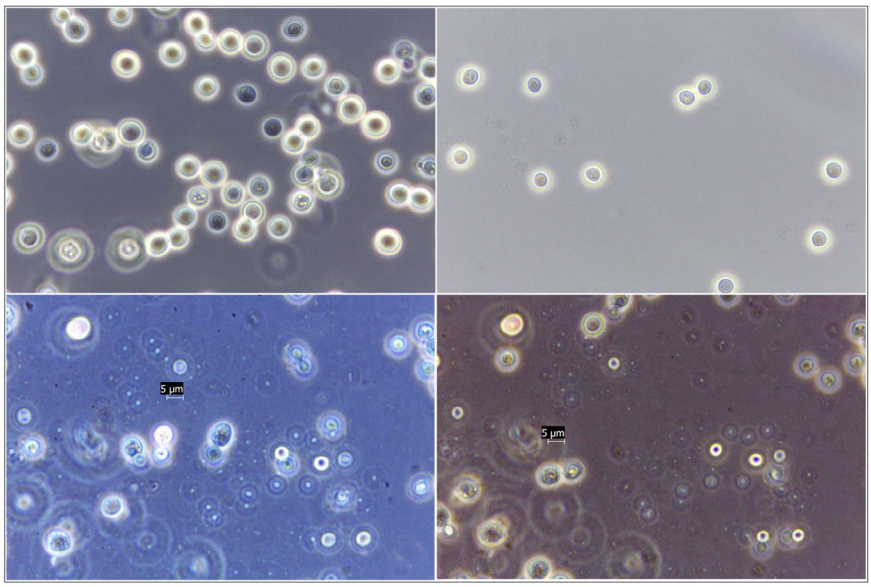

Fig. 9. Phase contrast microscopy of *Papiliotrema laurentii* under 100x magnification

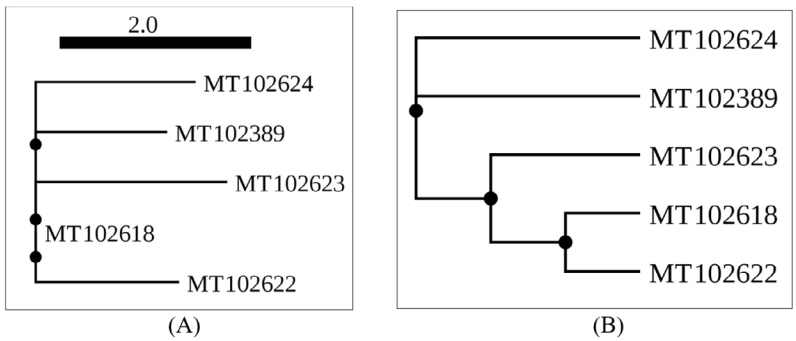

Fig. 10. (A) Phylogram and (B) Dendrogram prepared by using PhyML version: 3.0

Table 7. Sequence data of Cryptococcus species isolated from different sources.

Sl No	Laboratory Code	Source	Spacies	Accession identified (isolates)	Sequence (in FASTA format) No
1	GLP 19	Pigeon (Columba livia) dropping	Naganishia albida (Cryptococcus albidus)	MT 102389	>MT102389.1 Naganishia albida isolate GLP19 small subunit ribosomal RNA gene, partial sequence TTCTGGTGCCCAAGCAGCCGCGGTAATTCCAGCTCCAAT TAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTA GTTGAACTTCAGGCCCGACGGGGTGGTCTGCCTCACGG TATGTACTATCCGGTTGGGCCTTACCTCCTGGTGAGCC CGTATGTCGTTTACTCGGTGTGCGGGGGAACCAGGA ATTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGG CATATGCCCGAATACATTAGCATGGAATAATAGAATAG GACGTGCGGTTCTATTTTGTTGGTTTCTAGGATCGCC GTAATGATTAATAGGGACGGTTGGGGGCATTAGTATT CAGTTGCTAGAGGTGAAATTCTTAGATTTACTGAAG ACTAACTACTGCGAAAGCATTTGCCAAGGACGTTTTC ATTAATCAAGAACGAAGGTTAGGGGATCAAAAATGAT TAGATACCGTTGTAGTCCTAACAGTAAACTATGCCGA CTAGGGATCGGGCCATGTTCAACTTTTGACTGGCTCGG CACCTTACGAGAAATCAAAGTCTTTGGGTTCTGGGGG GAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGA CGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTT AATTTGACTCAACACGGGGAAACTCACCAGGTCCA GACATAGTAAGGATTGACAGATTGATAGCTCTTTCTTGAT TCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAG TGATTTGTCTGGTTTAATTCCGATAACGAAACGAGACCTTT AACCTTGCGTTAGAATTAGGACCCCGGGTTCAGGGGACCT
2	DHB 39	Evironmental sample near pigeons' dovecotes (contaminated soil)	Papiliotrema laurentii (Cryptococcus laurentii)	MT 102618	>MT102618.1 Papiliotrema laurentii isolate DHB39 small subunit ribosomal RNA gene, partial sequence TTTCTGGTGCCGCAGCAGCCGCGGTAATTCCAGCTCC AGTAGCGTATATTAAAGTTGCTTGCAGTTAAAAAGCTA GTAGTCGAACTTCGGCCCTGGCTGGACGGTCCGCCTTAC GGTGTGCACTGTCCGGCCGGGTCTTACCCTCTTGGTGAGG CCGCATGCCCTTTACTGGGTGTGCGGTGGAACCAGG AATTTTACCTTGAGAAAATTAGAGTGTTCAAAGCAGGCATTT GCCCGAATACATTAGCACGGAATAATAGAATAGGACGTGCGG TTCTATTTTGTTGGTTTCTAGGATCGCCGTAATGATTAATAG GGACGGTCGGGGGCATTAGTATTCCGTTGCTAGAGGTGAA ATTTTTAGATTTACGGAAGACTAACTTCTGCGAAAGCATTTGC CAAGGACGTTTTCATTGATCAAGAACGAAGGTTAGGGGATCA AAAACGATTAGATACCGTTGTAGTCTTAACAGTAAACTATGCC GACTAGGGATCGGGCCACGTTAATTTCTGACTGGCTCGGCACC TTACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTC GCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCAC CAGGTGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGG AAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATT GATAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCG TTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGA TAACGAACGAGACCTTAACCTGCTAAATAGCCAGGCCGG CTTTGGCTGGTTCCGGTACTTCTTTTACTGGACTGTACGT CTATTATACCCACACTGGATCTGGGACTAACCGCCGGTC GCTAGATCCCCCCTATGAGA
3	KRP 21	Dry barks of eucalyptus tree (Eucalyptus camaldulensis)	Papiliotrema laurentii (Cryptococcus laurentii)	MT 102622	>MT102622.1 Papiliotrema laurentii isolate KRP21 small subunit ribosomal RNA gene, partial sequence TTCCCGCTGCTGCTGCCCGCCCCGCACGCGGCAATCCAGCTC CAGTAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTA GTCGAACTTCGGGCCTGGCTGGACGGTCCGCCTTACGGTGT GCACTGTCCGGCCGGGTCTTACCTCTTGGTGAGGCCGCATG CCCTTTACTGGGTGTGCGGTGGAACCAGGAATTTTACCTTGA GAAAATTAGAGTGTTCAAAGCAGGCATTTGCCCGAATACATTA GCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGG TTTCTAGGATCGCCGTAATGATTAATAGGGACGGTCGGGGG CATTAGTATTCCGTTGCTAGAGGTGAAATTCTTAGATTTACGG AAGACTAACTTCTGCGAAAGCATTTGCCAAGGACGTTTTCAT TGATCAAGAACGAAGGTTAGGGGATCAAAAACGATTAGATA CCGTTGTAGTCTTAACAGTAAACTATGCCGACTAGGGATCGG GCCACGTTAATTTCTGACTGGCTCGGCACCTTACGAGAAATCA AAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAA

					ACTTAAAGGAATTGACGGAAGGGCACCACCAGGTGTGGAG CCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAG GTCCAGACATAGTAAGGATTGACAGATTGATAGCTCTTTC TTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGT GGAGTGATTTGTCTGGTTAATTCCGATAACGAACGAGACCTTAA CCTGCTAAATAGCCAGGCCGGCTTTGGCTGGTCGTCGGCTT CTTAGAGGGACTGTCGGCGTTTAGCCGACGGAGTTTGAG CAATCACAGATATAA
4	BNG 4	Pigeon (Columba livia) dropping	Papiliotrema laurentii (Cryptococcus laurentii)	MT 102623	>MT102623.1 Papiliotrema laurentii isolate BNG4 small subunit ribosomal RNA gene, partial sequence GCGGTAATTCCAGCTCCAGTAGCGTATATTAAAGTTGTTGCAG TTAAAAGCTCGTAGTCGAACTTCGGGCCTGGCTGGACGGT CCGCCTTACGGTGTGCACTGTCCGGCCGGGTCTTACCTCTT GGTGAGGCCGCATGCCCTTTACTGGGTGTGCGGTGGAACC AGGAATTTTACCTTGAGAAAATTAGAGTGTTCAAAGCAGG CATTTGCCCGAATACATTAGCATGGAATAATAGAATAGGAC GTGCGGTTCTATTTTGTTGGTTTCTAGGATCGCCGTAATGA TTAATAGGGACGGTCGGGGGCATTAGTATTCCGTTGCTAGA GGTGAAATTCTTAGATTTACGGAAGACTAACTTCTGCGAAA GCATTTGCCAAGGACGTTTTCATTGATCAAGAACGAAGGTT AGGGGATCAAAAACGATTAGATACCGTTGTAGTCTTAACAG TAAACTATGCCGACTAGGGATCGGGCCACGTTAATTTCTGAC TGGCTCGGCACCTTACGAGAAATCAAAGTCTTTGGGTTCTGG GGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGAC GGAAGGGCACCACCAGGTGTGGAGCCTGCGGCTTAATTTG ACTCAACACGGGGAAACTCACCAGGTCCAGACATAGTAAG GATTGACAGATTGATAGCTCTTTCTTGATTCTATGGGTGG TGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTA ATTCCGATAACGAACGAGACCTTAACCTGCTAAATAGCCA GGCCGGCTTTGGCTGGTCGTCGGCTTCTAGAGGGACGT CGGCGTTTAGCCGACGGAAGTTTGAGGCAATAACA
5	KJR 27	Pigeon (Columba livia) dropping	Naganishia albida (Cryptococcus albidus)	MT 102624	>MT102624.1 Naganishia albida isolate KJR27 small subunit ribosomal RNA gene, partial sequence AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAA GCTCGTAGTTGAACTTCAGGCCCGACGGGGTGGTCTGCCT CACGGTATGTACTATCCGGTTGGGCCTTACCTCCTGGTGAG CCCGTATGTCGTTTACTCGGTGTGCGGGGGAACCAGGA ATTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCATAT GCCCGAATACATTAGCATGGAATAATAGAATAGGACGTGCG GTTCTATTTTGTTGGTTTCTAGGATCGCCGTAATGATTAATA GGGACGGTTGGGGGCATTAGTATTCAGTTGCTAGAGGTGA AATTCTTAGATTTACTGAAGACTAACTACTGCGAAAGCATT TGCCAAGGACGTTTTCATTAATCAAGAACGAAGGTTAGGG GATCAAAAATGATTAGATACCGTTGTAGTCCTAACAGTAAA CTATGCCGACTAGGGATCGGGCCATGTTCAACTTTTGACTG GCTCGGCACCTTACAAAAAATCAAATTCTTTGGGTTCTGG GGGAAGTATGGTCGCAAGGCTGAACCTTAAAAGAATTGAC GGAGGGGCACCACCAGGCTTGAAGCCGGCGGCTTATTTTA ACTCATCACGGGGAAACTCACCAGGTCCGACACATACTAG GATTGACACATTGATATTTCTTTCTTGATTCTCTGGGTGGCGGTG CATGCCCGTTCTAAGTTGGTGGAGTGATTTGTCTGGTTAATG CCCAATCACGAACGTACATCTTAACCTGCTAACTACACCGA TCGGGCTTTGAGCTGCACCCTCTATTCTTTA

of health status of man and animals, these observations may be considered important as the prevalence of cryptococcosis is currently at a rising trend and an increase in the cases of fungemia both in human and animals have been on the rise in the past few years. However, in the current investigation, not a single isolate of *Cryptococcus neoformans* was recovered despite our incessant attempts to culture the isolate in appropriate Bird Seed Agar and Sabouraud Dextrose Agar media as per international standards.

The overall prevalence of *Cryptococcus spp.* was 15.40% (67) retrieved from all six geographical regions studied. Of the 67 positive isolates, 9.43% (41) and 5.98% (26) were identified to represent *Naganishia albida* and *Papiliotrema laurentii* respectively. The results are comparable with other findings reported by Jang *et al* (2011)[26] who stated the occurrence of 14.30% *Cryptococcus albidus* and 7.9% *Cryptococcus laurentii* in pigeon droppings from China. In another study, Kamari *et.al,* (2017)[16] reported 33

of 186 (17.7%) positive cultures and 11 out of 88 (12.5%) confirmed cultures of *Cryptococcus* spp. retrieved from pigeon nests and eucalyptus tree specimens from Ilam province in Iran. The authors also documented the prevalence of *Cryptococcus albidus* (17.2%), *C. albidus var. kuetzingii* (3.4%), *C. adilensis* (3.4%), *C. uzbekistanensis* (3.4%) and *C. neoformans var grubii* (3%) from pigeon nests while they concluded the presence of only *C. adilensis* (25%) in specimens from eucalyptus trees. .

The finding of 23 (14.11%) *Cryptococcus* in pigeon droppings from the dovecotes / houses of pigeon fanciers was comparable with the study of Kamari *et. al*, (2017)[16]. Our findings revealed 21 (10.34%) positive isolates of *Papiliotrema laurentii* in the droppings of pigeon in dovecotes / fanciers whereby 4 out of 51 (7.84%) and 1 (1.96%) represented *Naganishia albida* and *Papiliotrema laurentii* respectively. Similarly, 20.60% positive isolation rate of *Cryptococcus* in 6 out of 186 pigeon droppings in attics was reported earlier by Kamari*et.al,* (2017). In this study, 38 out of 89 (15.73%) isolates from attics were positive for *Cryptococcus* spp. The slight variation of the findings might be due to differences of climate, humidity, temperature and other biotic or abiotic factors characteristic of both the countries that are geographically apart and separated by a huge landmass.

In the current study, 13.49% (34) and 9.13% (23) of *Naganishia albida* and *Papiliotrema laurentii* could be recovered from pigeon droppings from attic ventilations of old buildings, dovecotes houses of pigeon fanciers and from cloacal swabs. Earlier, Rosario *et. al.* (2009)[24] reported a lower isolation rate of 23 (6.9%) *Cryptococcus albidus* and 2 (0.6%) *Cryptococcus laurentii* from droppings of pigeons in Spain which may be due to differences in environmental and climatic conditions as mentioned above. Studies have also been reported from Nigeria whereby Pal (2015) [47], had isolated 16 representatives of *Cryptococcus neoformans* from environmental samples of pigeon droppings with a prevalence of 12.5%. In the present study, the prevalence of *Naganishia albida* (13.49%) and *Papiliotrema laurentii* (9.13%) in pigeon droppings and in eucalyptus tree specimens were comparable to previous reported findings, despite the absence of *Cryptococcus neoformans*.

Although *Cryptococcus neoformans* is regarded as the most commonly occuring species of the *Cryptococcus* family, its absence in probable specimens collected from this part of the country is quite intriguing and it reflects the status of overall persistence of the species under humid conditions exposed to abrupt change in temperatures, a feature commonly observed in the studied locations. . The effect of seasonal variations on the persistence of non-neoforman *Cryptococcus* isolates cannot be nullified and it suggest the need of proper screening through a robust molecular approach concomitant to the behavior and food sources of the birds questioned, not to mention

Table 8. Geographical co-ordinates and environmental niches wise frequency distribution of positive isolates of Cryptococcus species

Sl No.	Geographical region	Total Sample	Naganishia albida positive (nos.)	Papiliotrema laurentii positive (nos.)	Total positive sample (nos.)	Relative % in the region	Absolute % in the region
1	BONGAIGAON	59 (BNG 1-59)	6	5	11	18.64	2.53
2	KOKRAJHAR	53 (KJR 1-53)	6	3	9	16.98	2.06
3	DHUBRI	91 (DHB 1-91)	10	6	16	17.58	3.68
4	KAMRUP	75 (KRP 1-75)	6	4	10	13.34	2.99
5	GOALPARA	104 (GLP 1-104)	10	6	16	15.38	3.68
6	NALBARI	53 (NLB 1-53)	3	2	5	9.43	1.15
						P = 0.762 (>0.05) : NS	
		TOTAL = 435	Total = 41	Total = 26	67	15.04 %	

NS: indicate non-significant

the presumable role of temperature and other edaphic factors that needs further appreciation.

Isolation of several species and varieties of the genus *Cryptococcus* from different sources, soils, atmospheric airs, dust and even from other avian species i.e chicken, ducks, parrots and munia had been previously reported by many authors. In a study conducted in Brazil, Leite *et. al,* (2012)[48] found the occurrence of 18 (21.4%) *Cryptococcus* species from 84 specimens of dust collected from public libraries. The isolation rate of *Cryptococcus albidus* 2 (4.6%) in their study was found to match our findings from dry soil 2 (4.88%). Furthermore, the percentage of isolation of *Cryptococcus* species in this study was 18.33% (58) as compared to 7.56% (9) from a total of 435 collected specimens in dry and moist conditions. The observations were in congruence with the number of isolates from pigeon droppings whereby 25.12% (51) and 12.24% (6) *Cryptococcus* isolates could be recovered from dry and moist specimens.

While studying the frequency of isolation in context of regional characteristics, no specific trend in terms of frequency was observed as the number of isolates varied from 9.43% (in Nalbari region) to 18.64% (Bongaigaon region), despite close proximity of the study areas that share the same environmental conditions. It may be recalled that factors like humidity, rainfall, temperature and pH of a definite geographical location do affect population parameters of domesticated birds like pigeons which need further evaluation. Ecological relevance of the non-neoformans species of *Cryptococcus* (4.84% in soil, 9.80% in eucalyptus tree samples) was the most important findings of this study with profound influence on replication and transmission of the pathogenic species from the ideal sources to the susceptible population at risk. Such situation is very imminent and highly dangerous for crowded and highly populated areas like Nalbari and Kamrup as the route of entry of such microorganisms is either through inhalation or direct skin contact.

From this investigation, it may be concluded that *Naganishia albida* and *Papiliotrema laurentii* are the most prevalent *Cryptococcus* species in the lower Assam belt compared to the more pathogenic *Cryptococcus neoformans* indicating the need of extensive epidemiological studies for establishing proper identify of the different strains that perpetuate, thrive and adapt to changing environmental conditions which are bound to challenge public health and pose as potential hazards in the near future. It may be stated that to ensure safety of the people, mass awareness programmes on the prevalence of the fungus need to be arranged whilst proper prevention, control and management of such disease causing pathogens be prioritized to prevent future outbreaks so as to minimize the risk of transmission of opportunistic *Cryptococcus* infections.

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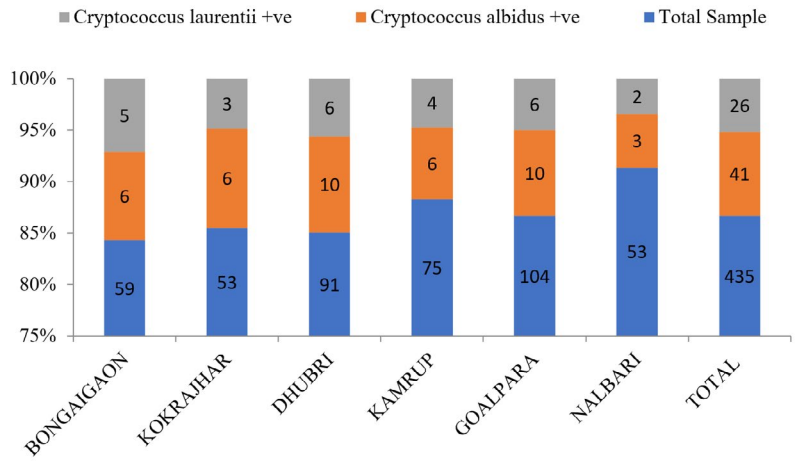

Fig. 11. District wise distribution of positive isolates of *Cryptococcus* species

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

As per the research design, data were collected from different primary sources / generated are available with the researcher and NCBI database (Accession No: MT 102389, MT 102618, MT 102622, MT 102623, MT 102624)

ETHICS STATEMENT

Not applicable. However, during the sample collection from pigeons, proper handling was done as per the standard veterinary practices.

REFERENCES

1. Lewis JL, Rabinovich S. The wide spectrum of cryptococcal infections. *Am J Med.* 1972;53(3):315-322. doi: 10.1016/0002-9343(72)90174-X.
2. Littman ML. Cryptococcosis (Torulosis). Current concepts and therapy. *Am J Med.* 1959;27(6):976-998. doi: 10.1016/0002-9343(59)90181-0.
3. Pal M, Dave P. Cryptococcosis: An Emerging Airborne Mycosis of Global Concern. *Air & Water Borne Diseases.* 2016;5(1):1000127. doi: 10.4172/2167-7719.1000127.
4. Kwon-Chung KJ, Boekhout T, Wickes BL, Fell JW. Systematics of the Genus *Cryptococcus* and Its Type Species *C. neoformans. Cryptococcus.* 2014.
5. Cheng MF, Chiou CC, Liu YC, Wang HZ, Hsieh KS. *Cryptococcus* laurentii fungemia in a premature neonate. *J Clin Microbiol.* 2001;39(4):1608-1611. doi: 10.1128/JCM.39.4.1608-1611.2001
6. Darze C, Lucena R, Gomes I, Melo, A. Clinical and laboratory characteristics of 104 Cryptococcus meningoencephalitis cases. *Revista Da Sociedade Brasileira de Medicina Tropical.* 2000.
7. Rosario I, Acosta B, Colom F. La paloma y otras aves como reservorio de *Cryptococcus* spp Pigeons and other birds as a reservoir for *Cryptococcus* spp. *Revista Iberoamericana de Micologia.* 2008;25(1):S13-S18. doi: 10.1016/S1130-1406(08)70020-2
8. Emmons CW. Isolation of *Cryptococcus neoformans* from soil. *J Bacteriol.* 1951;62(6):685-690. doi: 10.1128/JB.62.6.685-690.1951
9. Emmons CW. Prevalence of Cryptococcus neoformans in pigeon habitats. *Public Health Rep.* 1960:75:362-364. doi: 10.2307/4590800
10. Emmons CW. Saprophytic sources of *Cryptococcus* neoformans associated with the pigeon (*Columba livia*). *Am J Epidemiol.* 1955;62(3):227-232. doi: 10.1093/oxfordjournals.aje.a119775
11. Ruiz A, Velez D, Fromtling RA. Isolation of saprophytic *Cryptococcus neoformans* from Puerto Rico: Distribution and variety. *Mycopathologia.* 1989;106(3):167-170. doi: 10.1007/BF00443058
12. Fromtling RA, Abruzzo GK, Ruiz A. Virulence and antifungal susceptibility of environmental and clinical isolates of *Cryptococcus neoformans* from Puerto Rico. *Mycopathologia.* 1989;106(3):163-166. doi: 10.1007/BF00443057
13. Lazera MS, Cavalcanti MAS, Londero AT, Trilles L, Nishikawa MM, Wanke B. Possible primary ecological niche of *Cryptococcus neoformans*. *Med Mycol.* 2000;38(5):379-383. doi: 10.1080/mmy.38.5.379.383
14. Filiu WFdeO, WankeIII B, Aguena SM, et al. Cativeiro de aves como fonte de *Cryptococcus neoformans* na cidade de Campo Grande, Mato Grosso do Sul, Brasil. *Revista da Sociedade Brasileira de Medicina Tropical.* 2002;35(6). doi: 10.1590/S0037-86822002000600008
15. Kumar CPG, Prabu D, Mitani H, Mikami Y, Menon T. Environmental isolation of *Cryptococcus neoformans* and *Cryptococcus gattii* from living trees in Guindy National Park, Chennai, South India: Letter to the editor. *Mycoses.* 2010;53(3):262-264. doi: 10.1111/j.1439-0507.2009.01699.x
16. Kamari A, Sepahvand A, Mohammadi R. Isolation and molecular characterization of *Cryptococcus* species isolated from pigeon nests and Eucalyptus trees. *J Mycol Med.* 2017;3(2):20-25. doi: 10.29252/cmm.3.2.20
17. Pal M. *Cryptococcus gattii*: an emerging global mycotic pathogen of humans and animals. *J Mycopathol Res.* 2014.
18. Pal M, Randhawa HS. Caprine mastitis due to *cryptococcus neoformans. Med Mycol.* 1976;14(3):261-263. doi: 10.1080/00362177685190391
19. Pal M, Mehrotra BS. Ciyptococcal Mastitis in Daily Animals/Cryptococcen-Mastitis bei Milchvieh. *Mycoses.* 1983;26(12):615-616. doi: 10.1111/j.1439-0507.1983.tb03171.x
20. Pal M. First report of isolation of Cryptococcus neoformans var. neoformans from avian excreta in Kathmandu, Nepal. *Revista Iberoamericana de Micologia.* 1997.
21. Mitchell TG, Perfect JR. *Cryptococcosis* in the Era of

AIDS-100 Years after the Discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev.* 1995;8(4):515-548. doi: 10.1128/CMR.8.4.515-548.1995

22. Casadevall A, Mukherjee J, Scharff MD. Monoclonal antibody based ELISAs for cryptococcal polysaccharide. *J Immunol Methods*. 1992;154(1):27-35. doi: 10.1016/0022-1759(92)90209-C
23. Chander J, Sapra RK, Talwar P. Incidence of *cryptococcosis* in and around Chandigarh, India, during the period 1982-91. *Mycoses.* 1994;37:23-26. doi: 10.1111/j.1439-0507.1994.tb00280.x
24. Rosario I, de Mendoza MH, Deniz S, Soro G, Alamo I, Acosta B. Isolation of *Cryptococcus* species including *C. neoformans* from cloaca of pigeons. *Mycoses.* 2005:48(6):421-424. doi: 10.1111/j.1439-0507.2005.01153.x
25. Bernardo FM, Martins HM, Martins L. Fontes urbanas de Cryptococcus spp - Lisboa Urban Sources of Cryptococcus spp - Lisbon (Portugal). *Revista Portuguesa de Ciencias Veterinarias.* 2001.
26. Jang YH, Lee SJ, Lee JH, Chae HS, Kim SH, Choe NH. Prevalence of yeast-like fungi and evaluation of several virulence factors from feral pigeons in Seoul, Korea. *Lett Appl Microbiol.* 2011;52(4):367- 371. doi: 10.1111/j.1472-765X.2011.03009.x
27. Liu Y, Ma S, Wang X, Xu W, Tang J. *Cryptococcus albidus* encephalitis in newly diagnosed HIV-patient and literature review. *Med Mycol Case Rep.* 2014;3:8-10. doi: 10.1016/j.mmcr.2013.11.002
28. Huang YH, Lin IH, Chang TC, Tseng SH. Early diagnosis and successful treatment of *cryptococcus albidus* keratitis. *Medicine (United States).* 2015;94(19):e885. doi: 10.1097/MD.0000000000000885
29. Averbuch D, Boekhout T, Falk R, et al. Fungemia in a cancer patient caused by fluconazole-resistant *Cryptococcus laurentii*. *Med Mycol.* 2002;40(5):479-484. doi: 10.1080/mmy.40.5.479.484
30. Shankar EM, et al. Pneumonia and pleural effusion due to *Cryptococcus laurentii* in a clinically proven case of AIDS. *Can Respir J.* 2006;13(5):275-278. doi: 10.1155/2006/160451
31. von Geldern G, Nath A. Central nervous system infections. in Evidence-Based Neurology: Management of Neurological Disorders: Second Edition. 2015. doi: 10.1002/9781119067344.ch15
32. Criseo G, Bolignano MS, de Leo F, Staib F. Evidence of canary droppings as an important reservoir of *Cryptococcus neoformans*. *Zentralblatt fur Bakteriologie.* 1995;282(3):244-254. doi: 10.1016/S0934-8840(11)80124-6
33. Liu Z, Ma L, Zhong Y, Wang X, Xie S. Isolation, identification and significance of *Cryptococcus neoformans* and *Candida albicans* from faecal specimen of pigeon. *Advances in Intelligent and Soft Computing.* 2012;134:507-512. doi: 10.1007/978-3-642-27537-1_63
34. Grossgebauer K. Detection of *cryptococcus neoformans* capsule using fluorescent, mucopolysaccharide-binding dyes. *Klin Wochenschr.* 1980;58(18):943-945. doi: 10.1007/BF01477052
35. Chae HS, Park GN, Kim SH, et al. Rapid direct identification of *Cryptococcus neoformans* from pigeon droppings by nested PCR using CNLAC1 gene. *Poultry Science.* 2012;91(8):1983-1989. doi: 10.3382/ps.2012-02307
36. Filion T, Kidd S, Aguirre K. Isolation of *Cryptococcus laurentii* from Canada Goose guano in rural upstate New York. *Mycopathologia.* 2006;162(5):363-368. doi: 10.1007/s11046-006-0066-1
37. Mseddi F, Jarboui M, Sellami A, Sellami H, Ayadi A. A rapid and easy method for the DNA extraction from *Cryptococcus neoformans*. *Biol Proced Online.* 2011;13(1):5. doi: 10.1186/1480-9222-13-5
38. Tendulkar SR, Gupta A, Chattoo BB. A simple protocol for isolation of fungal DNA. *Biotechnol Lett.* 2003;25(22):1941-1944. doi: 10.1023/B:BILE.0000003990.27624.04
39. Motkova P, Vytrasova J. Comparison of methods for isolating fungal DNA. *Czech Journal of Food Sciences.* 2011;29:s76-s85. doi: 10.17221/266/2011-CJFS
40. Leal AL, Faganello J, Bassanesi MC, Vainstein MH. *Cryptococcus* species identification by multiplex PCR. *Med Mycol.* 2008;46(4):377-383. doi: 10.1080/13693780701824429
41. Lau A, Chen S, Sorrell T, et al. Development and clinical application of a panfungal PCR assay to detect and identify Fungal DNA in tissue specimens. *J Clin Microbiol.* 2007;45(2):380-385. doi: 10.1128/JCM.01862-06
42. Mitchell TG, Freedman EZ, White TJ, Taylor JW. Unique oligonucleotide primers in PCR for identification of *Cryptococcus neoformans*. *J Clin Microbiol.* 1994;32(1):253-255. doi: 10.1128/JCM.32.1.253-255.1994
43. Sidrim JJC, Costa ANF, Cordeiro RA, et al. Molecular methods for the diagnosis and characterization of *cryptococcus*: A review. *Can J Microbiol.* 2010;56(6):445-458. doi: 10.1139/W10-030
44. XLSTAT. Statistical Power for Repeated Measures Anova. Adinsoft. 2015.
45. Barbosa AM, Santos BFdeO, Carvalho EdEO, et al. Biological activity of *Cryptococcus neoformans* and *Cryptococcus gattii* from clinical and environmental isolates. *Jornal Brasileiro de Patologia e Medicina Laboratorial.* 2013;49(3). doi: 10.1590/S1676-24442013000300002
46. Adinsoft S. XLSTAT-software, version 10. Addinsoft, Paris, France. 2010.
47. Pal M. First Record of Isolation of *Cryptococcus neoformans* from Pigeon Droppings in Djibouti. *Molecular Microbiology Research.* 2015;5(3):1-3. doi: 10.5376/mmr.2015.05.0003
48. Leite DP, Amadio JVRS, Martins ER, et al. *Cryptococcus* spp isolated from dust microhabitat in Brazilian libraries. *Journal of Occupational Medicine and Toxicology.* 2012;7(1)11. doi: 10.1186/1745-6673-7-11