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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Decayed wood inside hollow trunks of living trees of *Tamarindus indica*, *Syzygium cumini* and *Mangifera indica* as natural habitat of *Cryptococcus neoformans* and their serotypes in Jabalpur City of Central India

Le bois pourri à l'intérieur des creux de tronc d'arbres vivants (*Tamarindus indica*, *Syzygium cumini* et *Mangifera indica*) habitat naturel de *Cryptococcus neoformans* et de ses sérotypes dans la ville de Jabalpur en Inde centrale

S.R. Nawange[a], K. Shakya[a], J. Naidu[b], S.M. Singh[a,*], N. Jharia[a], S. Garg[b]

[a] *Medical mycology laboratory, Flat 2 C, Pavitra Apartment, South Civil Lines, department of biological science, Rani-Durgavati university, Jabalpur 482001, M.P., India*
[b] *Biotechnology laboratory, department of zoology, government science college, Jabalpur 482001, M.P., India*

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KEYWORDS
Natural habitat;
Cryptococcus gattii;
Cryptococcus neoformans var. *grubii*

Abstract

Objective. – Colonization of *Cryptococcus gattii* and *Cryptococcus neoformans* var. *grubii* inside the decaying wood of hollow trunks of *Tamarindus indica*, *Syzygium cumini* and *Mangifera indica* is reported in Jabalpur City in Central India.

Methods. – The decayed wood inside the hollows trunks of 133 trees mostly belonging to the three species *T. indica*, *S. cumini* and *M. indica* was examined. The 108 samples performed during the 1010 days monitoring period were inoculated on Staib's medium. The strains of *C. neoformans* isolated were identified by morphological, biochemical and serological characters.

* Corresponding author.
E-mail addresses: sr_nawange@yahoo.com (S.R. Nawange), smsingh_rdvv@rediffmail.com (S.M. Singh).

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Results. – Four out of 36 (11.11%) *T. indica* trees investigated proved to be positive for *C. gattii* (serotype B) and three out of 36 (8.33%) *T. indica* trees were positive for *C. neoformans* var. *grubii* (serotype A). Two out of 30 (6.66%) *M. indica* trees were found to be positive for *C. gattii* serotype B and one of 30 (3.33%) was positive for *C. neoformans* var. *grubii* serotype A. Of 42 *S. cumini* trees, four (9.52%) were found to be positive for *C. gattii* serotype B and two (4.76%) for *C. neoformans* var. *grubii* serotype A. The two varieties never co-occurred in the same hollow of any of the trees investigated. The data strongly support the colonization of *T. indica*, *M. indica* and *S. cumini* trees by both varieties of *C. neoformans*. Evidence of this was found by repeated isolations and by the high population density in the decayed wood during the 1010 days monitoring period. *M. indica* is reported, for the first time, as the natural habitat of both varieties of *C. neoformans*. For the first time *T. indica* is also reported to harbor *C. gattii* serotype B.
Conclusion. – Our results further reinforce recently emerging evidence that the natural habitat of *C. gattii* and *C. neoformans* var. *grubii* is more generalized than specific.

MOTS CLÉS
Habitat naturel;
Cryptococcus gattii;
Cryptococcus neoformans var. *grubii*

Résumé
Objectif. – La colonisation de *Cryptococcus gattii* et *Cryptococcus neoformans* var. *grubii* à l'intérieur du bois pourri des creux de tronc d'arbres vivants de *Tamarindus indica*, *Syzygium cumini* et *Mangifera indica* est rapportée dans la ville de Jabalpur en Inde centrale.
Méthodes. – Le bois pourri à l'intérieur des creux des troncs de 133 arbres appartenant aux trois espèces *T. indica*, *S. cumini* et *M. indica* a été examiné. Les 108 prélèvements effectués sur une période de 1010 jours ont été mis en culture sur milieu de Staib. Les souches de *C. neoformans* isolées ont été identifiées par leurs caractères morphologiques, biochimiques et sérologiques.
Résultats. – Quatre des 36 (11,11 %) *T. indica* étudiés ont été positifs pour *C. gattii* sérotype B et 3 sur 36 (8,33 %) ont été positifs pour *C. neoformans* var. *grubii* sérotype A. Deux des 30 (6,66 %) *M. indica* ont été trouvés positifs pour *C. gattii* sérotype B et 1 sur 30 (3.33 %) a été positif pour *C. neoformans* var. *grubii* sérotype A. Des 42 *S. cumini*, quatre (9,52 %) ont été trouvés positif pour *C. gattii* sérotype B et deux (4,76 %) pour *C. neoformans* var. *grubii* sérotype A. Les deux variétés n'ont jamais été observées ensemble dans la même cavité de tous les arbres étudiés. Les données suggèrent fortement la colonisation de *T. indica, M. indica* et *S. cumini* par les deux variétés de *C. neoformans*. *M. indica* est rapporté, pour la première fois, comme habitat normal des deux variétés de *C. neoformans*. En outre, *T. indica* est également rapporté, pour la première fois, comme étant porteur de *C. gattii* sérotype B.
Conclusion. – Nos résultats renforcent l'argument récent que l'habitat normal de *C. gattii* et *C. neoformans* var. *grubii* est plus généralisé que spécifique.

Introduction

Ecology of *Cryptococcus neoformans* has been the subject of interest since first description of a case of cryptococcosis [3]. In the same year Sanfelice [25] isolated the fungus from peach juice, and was named as *Saccharomyces neoformans*. The fungus was also isolated from a peach fruit collected from a local market in Berlin [26,28–30]. Although *C. neoformans* variety *neoformans* was first isolated from peach juice, the most important natural sources known to date is weathered dropping from pigeons (*Columbia livia*) and soil contaminated with avian droppings [2,8]. In 1986, Bauwens et al. [4] reported *C. neoformans* var. *neoformans* from outside and inside of trunk hollows within an aviary. This study suggested that some trees can provide a natural habitat for *C. neoformans* var. *neoformans*. In 1990, Ellis and Pfeiffer [7], from Australia reported that plant debris under the canopies of *Eucalyptus camaldulensis* trees can be a source of *Cryptococcus gattii* serotype B. These observations were later extended to *E. tereticornis* in Australia. Pfeiffer and Ellis [20,21] later isolated *C. gattii* from *E. camaldulensis* trees in San Francisco and California. These reports stimulated a number of studies focusing on decaying wood in trunk hollows of several living trees. Till date, 23 tree species belonging to diverse families have been reported as natural habitat of *C. neoformans* varieties [5,9,11,15–18,21–24,31]. In India, Randhawa et al. [23] reported the isolation of *C. neoformans* var. *grubii* from decayed wood debris collected from trunk hollows of *Butea monosperma* and *Tamarindus indica* from Delhi and New Delhi and the bark of a *Eucalyptus* tree in Amritsar. In 2003, Randhawa et al. [22] reported the isolation of *C. neoformans* var. *grubii* and *C. gattii* from trunk hollow of *Syzygium cumini* and *Ficus religiosa*. Recently there has been some changes to the taxonomy of *Cryptococcus* Kwon-Chung et al. [13] elevated *C. neoformans* var. *gattii* to species level as *C. gattii*. Likewise, Franzot et al. [10] have proposed *C. neoformans* var. *grubii* for *C. neoformans* var.

neoformans serotype A isolates. In present paper the current taxonomic trend has been followed.

In the present paper, we report the isolation of *C. neoformans* var. *grubii* and *C. gattii* from decayed wood inside trunk hollows of *T. indica*, *S. cumini* and *Mangifera indica* trees growing in Jabalpur City of Central India.

Materials and methods

Study area: Jabalpur, a district headquarter of Madhya Pradesh (Central India) is located more or less in the Center of India (Fig. 1a,b). It is situated at 23°10′ latitude and 79°57′ E longitude with altitude of 393 m above mean sea level. Its

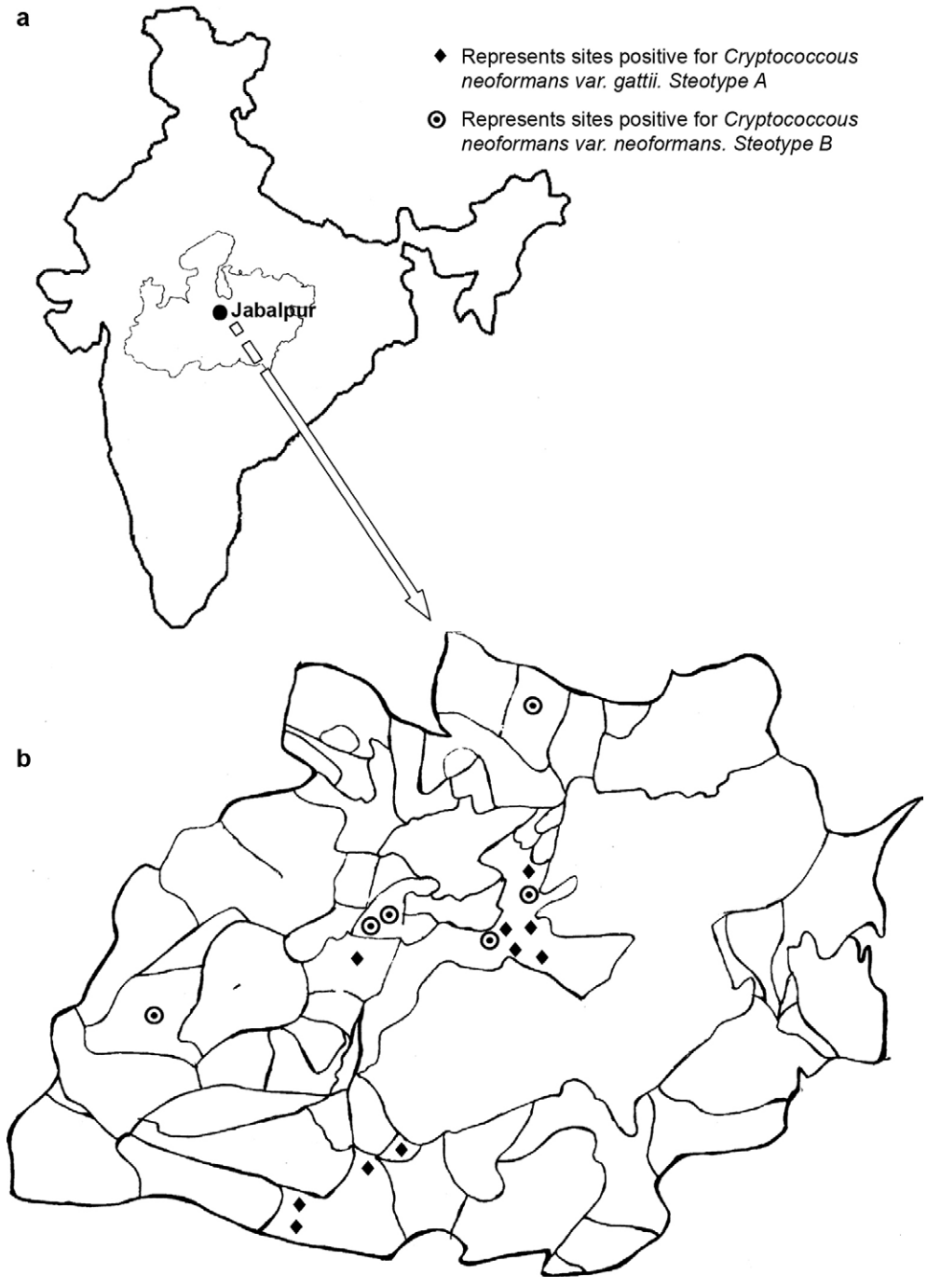

Figure 1a Map of India showing the location of Jabalpur City.
Figure 1a Carte de l'Inde montrant la localisation de Jabalpur.
Figure 1b Map of Jabalpur City in the state of Madhya Pradesh, Central India, showing location of trees positive for *C. neoformans* var. *grubii* and *C. gattii*. TI: *T. indica*, MI: *M. indica*, SC: *S. cumini*.
Figure 1b Carte de Jabalpur dans l'état de Madhya Pradesh, Inde centrale, montrant l'emplacement des arbres positifs à *C. neoformans* var. *grubii* and *C. gattii*.

area is 134.70 km^2 and population about 1.5 million. Climatically it is divided into summer, rainy and winter seasons. In summer the temperature may go up to 45 °C and in winter 6 °C. Average yearly rainfall is 50 in. and relative humidity maximum 77.73, minimum 41.55.

Sample collection

Decaying wood of trunk hollows of 133 living trees growing in Jabalpur City of Central India were investigated for the presence of *C. neoformans*. These were 36 *T. indica* trees (family *Caesalpiniaceae*); 30 *M. indica* (family *Anacardiaceae*); 42 *S. cumini* (family *Myrtaceae*); 10 *Madhuca indica* (family *Sapotaceae*); 10 *Ficus racemosa* (family *Moraceae*) and five *Acacia nilotica* (family *Mimosaceae*). A total of 108 samples of the above trees were examined for *C. neoformans*. These trees were selected on the basis of having hollows in there main trunk and were growing along the road sides and public places in the city. No *Eucalyptus* trees were seen growing near the trees investigated. No avian excreta were traced in the trunk hollows. The samples were collected from January 2002 to December 2004. Decaying wood pieces from inside trunk hollows were aseptically collected with long forceps and stored in clean sterilized polythene bags. The samples were taken to laboratory and processed. In case of delay they were stored at room temperature and processed within 48 hours.

Processing of the sample

About 5 g of trunk hollows wood debris sample was suspended in 45 ml sterilized physiological saline containing streptomycin sulfate (40 μg/ml) and penicillin (20 μg/ml). The suspension was intermittently shaken for 5 min and then allowed to settle at 37 °C for 1 hour. The supernatant was collected with a sterile pasture pipette. Aliquot of 0.1 ml of supernatant as well as of 1:10, 1:100, 1:1000 dilution of the suspension in sterile water were streaked in triplicate on plates of Staib's niger seed agar medium of the following composition: Niger seed 70 g, glucose 10 g, agar 20 g, KH_2 PO_4 1 g, creatinine 1 g, streptomycin sulfate 40 μg/ml, penicillin 20 μg/ml and diphenyl solution (1 g/10 ml 95% alcohol) 10 ml, and distilled water 1000 ml [12]. The inoculated plates were incubated at 28 °C and examined daily for 8 days. Yeast like mucoid colonies showing brown pigment were examined microscopically and iso-

Table 1 Natural occurrence of *C. neoformans* var. *grubii* (*CnVG*) and *C. gattii* (*Cg*) from decaying wood in hollow (espace) trunks of *T. indica*, *M. indica*, *S. cumini* living trees in city of Jabalpur in Central India
Tableau 1 Habitat naturel de *C. neoformans* var. *grubii* (CnVG) et de *C. gattii* (Cg) dans le bois pourri des creux de tronc de *T. indica*, *M. indica* et *S. cumini* dans la ville de Jabalpur en Inde Centrale

Type of samples/tree species	Locality/place		Date of collection	Number of trees positive/ examined	Number of trees yielding	
					*C.n.*var. *grubii* (*CnVG*)	*C. gattii* (*Cg*)
					Serotype A	Serotype B
Wood debris inside a tree trunk hollows of *T. indica* (the tamarind tree family Caesalpiniaceae)	Gaurighat Road, Jabalpur	TI 15	15th March 2002	1\5	0	1
	Gaurighat Road, Jabalpur	TI 31	18th January 2002	1\5	0	1
	Rampur Chowraha, Jabalpur	TI 42	15th April 2002	1\3	0	1
	Tahsil Chowk, Jabalpur	TI 52	15 February 2002	1\6	0	1
	Gantaghar Jabalpur	TI 58, TI 102	10 May 2003	2\10	2	0
	Around Medical College campus Jabalpur	TI 60	20 June 2003	1\7	1	0
Wood debris inside a tree trunk hollows of *M. indica* (the mango tree family Anacardiaceae)	Wright Town Bus Stand, Jabalpur	MI 105	24th March 2002	1\6	0	1
	Civil Line Station Road, Jabalpur	MI 110	25 May 03	1\8	1	0
	Katanga Road, Jabalpur	MI 120	20 June 2003	1\8	0	1
	Bhedhaghat Road, Jabalpur		17 February 2004	0\8	0	0
Wood debris inside a tree trunk hollows of *S. cumini* (the Indian black berry, black plum, Java plum, family Myrtaceae)	Veterinary College, Civil Line, Jabalpur	SC 25	11th February 2002	1\6	0	1
	Sihora Road, Jabalpur	SC 77	10 May 2003	1\12	1	0
	Civil Line, Jabalpur	SC 78,SC80	25th March 2004	2\10	0	2
	Civil Line, University Road Jabalpur	SC90	20 December 2003	1\9	1	0
	Civil Line Station Road, Jabalpur	SC65	10 November 2003	1\5	0	1
			Total	16/108	6	10

lated in pure culture by dilution plating. Identification was based on morphological and physiological characteristics [12,14]. The variety of *C. neoformans* was ascertained by canavanine glycine bromothymol blue (CGB) medium [14]. Serotyping of the isolates identified as *C. gattii* and *C. neoformans* var. *grubii* was done by the slide agglutination test based on monoclonal antibody (CRYPTO CHECK 25 Test kit, Iatron Laboratories Inc., Chiyoda-Ku, Tokyo, Japan).

Results

Out of six tree species investigated, three tree species viz. *T. indica*, *M. indica* and *S. cumini* were found to grow *C. neoformans* var. *grubii* and *C. gattii* (Table 1). These isolates were found in decaying wood inside trunk hollows (Figs. 2, 3 and 4). Seven of the 36 (19.44%) *T. indica*, three of 30 (10%) *M. indica* and six of 42 (14.28%) *S. cumini* trees were positive for *C. gattii* and *C. neoformans* var. *grubii*. Two out of 10 and one of seven *T. indica* trees growing at Gantaghar and Medical College areas, respectively, yielded *C. neoformans* var. *grubii* serotype A. Out of 19 *T. indica* trees investigated in the area including that of Gawarighat, Rampur Chowk, and Tehsil Chowk, four yielded *C. gattii* serotype B. None of the trees revealed mixed colonization by *C. neoformans* var. *grubii* and *C. gattii*. Similarly three trees of *M. indica* positive for *C. gattii* and *C. neoformans* var. *grubii* were located on the sides of thickly populated areas of Jabalpur City. Out of six trees of *M. indica* investigated, one tree located in Wright Town yielded *C. gattii* serotype B. Similarly, out of eight *M. indica* trees investigated, one yielded *C. gattii* serotype B on the Katanga road side. The remaining one *M. indica* tree positive for *C. neoformans* var. *grubii* serotype A was located in the Civil Lines area. Regarding *S. cumini*, of six positive trees for *C. gattii* and *C. neoformans* var. *grubii*, four were found harboring *C. gattii* serotype B and from remaining two trees *C. neoformans* var. *grubii* serotype A was isolated (Table 1).

Out of four repeatedly sampled *T. indica* trees harboring *C. gattii* trees, TI-15, TI-31, TI-42 and TI-52 were positive for the pathogen on 3/6, 3/7, 2/4 and 2/5 occasions, respectively, over a period up to 1010 days follow-up period. For *C. neoformans* var. *grubii* TI-58 was positive on all five occasions when testing was done, whereas TI-102 and TI-60 of *T. indica* trees yielded repeated isolations on 3/5 and 4/5 occasions when testing was done up to 300 days follow-up period.

Out of two repeatedly sampled *M. indica* trees harboring *C. gattii*, trees MI-105 and MI-120 were positive for the fungus on 2/5 and 3/4 occasions repeatedly up to 626 days follow-up period in case of MI-105 and 241 days follow-up

Figure 2 Close up of *S. cumini* (SC-77). Decaying wood inside its trunk hollows was found positive for *C. neoformans* var. *grubii*.
Figure 2 Détail de *S. cumini* (SC-77). Le bois pourri à l'intérieur du tronc s'est révélé positif pour *C. neoformans* var. *grubii*.

Figure 3 Close up of *T. indica* (TI-15). Decaying wood inside its trunk hollows was found positive for *C. gattii*.
Figure 3 Détail de *T. indica* (TI-15). Le bois pourri à l'intérieur du tronc s'est révélé positif pour *C. gattii*.

Figure 4 Close up of *M. indica* (MI-120). Decaying wood inside its trunk hollows was found positive for *C. gattii*.
Figure 4 Détail de *M. indica* (MI-120). Le bois pourri à l'intérieur du tronc s'est révélé positif pour *C. gattii*.

in case of MI-120. Tree MI-110 was positive on all the occasion when testing was done for *C. neoformans* var. *grubii* (Table 2). Of the four repeatedly sampled *S. cumini* trees known to harbor *C. gattii*, trees SC-78, SC-80 were positive for *C. gattii* on all occasions tested. Similarly, tree SC-77 and SC-90 were positive on all occasions for *C. neoformans* var. *grubii* when testing was done. However, trees SC-25 and SC-65 had positive cultures for *C. gattii* on 2/6 and 3/4 occasions tested over a period up to 599 days follow-up (Table 2).

It was noted, in the present study, that population densities of *C. gattii* and *C. neoformans* var. *grubii* in the trees investigated showed wide quantitative variations from season to season and from tree to tree (Table 2). In the trunk hollows of *T. indica* lowest density 4×10^3 cfu/g of *C. neoformans* var. *grubii* was seen in TI-58 in the month of February 2004 and highest was 2×10^5 cfu/g in TI-120 in the month of April. Similarly lowest density of *C. gattii* in the trunk hollow of *M. indica* was1.5×10^3 cfu/g seen in November 2003 in the tree MI-120 and the highest density, i.e. 6×10^5 cfu/g was seen in MI-105 tree of *M. indica* in December 2003. In the trunk hollow of *S. cumini* the lowest density, i.e. 1.5×10^4 cfu/g *C. gattii* was noted in the tree SC-78 during March to May 2004 and highest density, i.e. 5×10^4 cfu/g of *C. gattii* was found in the tree SC-80 (Table 2).

All samples obtained from the trunk hollows of the living trees of *Madhuca indica*, *F. racemosa* and *A. nilotica* were found negative for *C. neoformans*.

Discussion

The results of the present study has revealed that decaying wood in trunk hollows of living *T. indica*, *S. cumini* and *M. indica* trees serves as a natural habitat for *C. gattii* and *C. neoformans* var. *grubii*. Previously in India, Randhawa et al. [23] have found *T. indica* tree to harbor only *C. neoformans* var. *grubii* serotype A. However, the present study has revealed that this tree in addition to *C. neoformans* var. *grubii* serotype A, could also harbor *C. gattii* serotype B. Similarly, the present study also reinforce the finding of Randhawa et al. [22] that *S. cumini* tree could inhabit both the varieties of *C. gattii* and *C. neoformans* var. *grubii*. To our knowledge, the association of *C. gattii* with *T. indica* revealed by the present study has not been reported previously. In addition, the present study has also revealed for the first time, that *M. indica* a common mango tree in India, harbors both the varieties of *C. gattii* and *C. neoformans* var. *grubii*.

In India, Chakrabarti et al. [6] have reported the isolation of *C. gattii* serotype B from the flower of *E. camaldulensis* tree in Punjab but the number of colonies isolated was not given by them. Similarly though Pfeiffer and Ellis [21] had found that flower and other plant debris of *E. camaldulensis* and *E. terticornis* constituted the main natural habitat of *C. gattii*, however, *C. gattii* has not been found in *Eucalyptus* sampling done in other parts of India [1,19]. In the present study, it has been found that trunk hollows of *T. indica, S. cumni* and *M. indica* are the most important natural habitat for *C. gattii* serotype B as well as of *C. neoformans* var. *grubii* serotype A as suggested by the presence of their population density in Jabalpur City and possibly in many other parts of India where these tree species grow. It was also interesting to note that none of the trunk hollows of all the positive trees yielded mixed population of *C. gattii* serotype B and *C. neoformans* var. *grubii* serotype A. Similar findings were also observed by Randhawa et al. [22] for *S. cumini* and *F. religiosa*.

C. gattii and *C. neoformans* var. *grubii* has been only rarely reported from plant sources in India [6,19]. Randhawa et al. [23] isolated *C. neoformans* var. *grubii* from trunk hollows of *B. monosperma* and *T. indica*. However they failed to reisolate the fungus from *B. monosperma* at a later date but could reisolate it from *T. indica* [22]. In the present study, however, we could isolate *C. gattii* and *C. neoformans* var. *grubii* repeatedly for 2 years from the trunk hollow of *T. indica*, *M. indica* and *S. cumini* suggesting their significance as natural source for both the varieties of *C. gattii* and *C. neoformans* var. *grubii*. It would, however, be worth investigating these trees for *C. gattii* and *C. neoformans* var. *grubii* in other parts of India as well to establish these trees as the natural ecological niche of the pathogen.

Randhawa et al. [22,23] successfully used simplified Staib's niger seed agar excluding creatinine and KH_2PO_4 for isolation of both varieties of *C. gattii* and *C. neoformans* var. *grubii* from environmental sources.

Table 2 Population density and (repeat) isolation of *C. gattii* (*Cg*) and *C. neoformans* var. *grubii* (*CnVG*) from decaying wood in trunks hollows of *T. indica*, *M. indica*, *S. cumini* living trees in city of Jabalpur in Central India
Tableau 2 Densité de population et isolement de *C. gattii* (Cg) et de *C. neoformans* var. *grubii* (CnVG) dans le bois pourri des creux de tronc de *T. indica*, *M. indica* et *S. cumini* dans la ville de Jabalpur en Inde Centrale

Tree species	Tree number	Samples positive/ number examined	Collection date of culture positive samples	Sampling date and cfu/g	Observation span (days)	Variety/ serotypes
T. indica	TI 15	3\6	15 March 2002; 2 April; 10 June; 10 September 2003; 17 March; 12 November2003	15 March 2002; 0.5×10^5	607	*C.g/B*
	TI 31	3\7	18 March 2002; 5 January; 18 March; 20 May; 25 August; 8 October 2003; 22 December 2004	18 March 2002; 0.6×10^5; 8 October 2003; 2.0×10^5	1010	*C.g/B*
	TI 42	3\4	15 April 2002; 16 September; 9 November 2003; 26 February 2004	15 April 2002; 0.8×10^5; 9 November 2003; 0.7×10^5; 26 February 2004; 3.0×10^4	682	*C.g/B*
	TI 52	2\5	15 February 2002; 12 April; 25 June; 12 August; 2003; 23 September 2003	15 February 2002; 0.7×10^5	585	*C.g/B*
	TI 58	5\5	10 May 2003; 15 September; 10 November 2003; 27 February 2004; 20 April 2004	10 May 2003; 8.0×10^3; 27 February 2004; 4.0×10^4	346	*CnVG/A*
	TI 102	3\5	11 April 2003; 19 June; 4 October 2003; 8 December 2003; 22 February 2004	11 April 2003; 2.0×10^5; 22 February 2004; 1.5×10^5	300	*CnVG/A*
	TI 60	4\5	20 June 2003; 9 August ; 15 September 2003; 7 December 2003;5 February 2004	20 June 2003; 1.4×10^4; 7 December 2003 1.9×10^4	200	*CnVG/A*
M. indica	MI 105	2\5	24 March 2002; 15 September; 2 November 2003; 10 December 2003	24 March 2002; 0.7×10^5; 10 December 2003; 6.0×10^5	626	*C.g/B*
	MI 110	4\4	25 May 2003; 12 September 2003;13 December 2003; 10 June 2004	25 May 2003; 2.0×10^4	381	*CnVG/A*
	MI 120	3\4	20 June 2003; 8 October 13 December 2003; 16 February 2004	20 June 2003; 3.0×10^4; 16 February 2004; 1.5×10^3	241	*C.g/B*
S. cumini	SC 25	2\6	11 February 2002; 5 January 2003; 16 March; 19 May; 20 July; 3 October 2003	11 February 2002; 0.7×10^5; 5 January 2003; 2.0×10^4; 3 October 2003; 1.5×10^5	599	*C.g/B*
	SC 77	5\5	10 May 2003; 20 July 5 October 2003; 8 December 2003; 7 February 2004	10 May 2003; 4.0×10^4; 8 December 2003; 3.0×10^4	273	*CnVG/A*
	SC 78	3\3	25 March, 2004; 10 April 2004; 12 May 2004	25 March 2004; 1.5×10^4; 12 May 2004; 1.5×10^4	58	*C.g/B*
	SC 80	3\3	25 March 2004; 10 April 2004; 15 May 2004	25 March 2004; 5.0×10^4; 15 May 2004; 3.0×10^4	51	*C.g/B*
	SC 90	4\4	20 December 2003; 5 January; 19 March 2004; 10 May 2004	20 December 2003; 2.5×10^4; 19 March 2004; 3.0×10^4; 10 May 2004; 1.8×10^4	141	*CnVG/A*
	SC 65	3\4	10 November 2003; 5 February 2004; 18 April 2004; 10 May 2004	10 November 2003; 2.0×10^3; 10 May 2004; 1.5×10^4	182	*C.g/B*

Cg: B denotes *C. gattii*; *CnVG*: A denotes *C. neoformans* var. *grubii*.

Staib [27], however, raised a question about the efficacy of the medium without creatinine for the isolation of *C. gattii* because *C. gattii* which utilized creatinine more efficiently can be best isolated in a creatinine supplemented medium. In the present study, we have used Staib's medium supplemented with creatinine and KH_2PO_4 and found it very effi-

Table 3 Distribution of *Cryptococcus neoformans* var. *grubii* (*CnVG*) and *Cryptococcus gattii (Cg)* according to host tree species
Tableau 3 Répartition de *Cryptococcus neoformans* var. *grubii* (*CnVG*) et de *Cryptococcus gattii (Cg)* selon les espèces d'arbres hôtes.

C. n. **var.** *grubii(CnVG)* **serotype A**	*C. gattii (Cg)* **serotype B**	*C.n.* **var.** *neoformans (CnVN)* **serotype C**	**Both varieties**
Butea monosperma, forest flame. abaceae [18]	*Angophora costata*, Sydney red gum. Myrtaceae [19]	*Terminalla catappa*, native almond tree. Combretaceae [17]	*Adenanthera povonina*, coral wood, redwood. Mimosaceae [19]
Caesalpinia peltophoroides, sibi-Piruna. Caesalpiniaceae [21]	*Erythrina velutina*, mulungo, Fabaceae [21]		*Cassia grandis*, pink shower tree. Caesal-Piniaceae [14]
Ficus religiosa, peepul, bo tree, odhi tree, Moraceae [18]	*Eucalyptus grandis*, flooded gum, rose gum. Myrtaceae [19]		*Eucalyptus camaldulensis*, red river gum. [19]
Miroxylon peruiferum, Peruvian Balsam. Fabaceae [21]	*E. haemostoma*, seribbly gum. Myrtaceae [19]		*Ficus microcarpa*, laurel fig, laurel fig, Indian laurel, Green Island fig, Chinese banyan. Moraceae [14]
Senna multijugga, November Shower tree. Caesalpiniaceae [14]	*E. microcorys*, tallow-wood. Myrtaceae [19]		*Syzygium cumini*, Indian black berry, black berry, Java plum. Myrtaceae [23]
Tamarindus indica, tamarind. Caesalpiniaceae [18]	*E. tereticornis*, forest red gum. Myrtaceae [12]		*Tamarindus indica, Syzygium cumini, Mangifera indica*, Mango tree. Sapotaceae [Present study]
Theobroma cacao, cacao, choco-Late tree, cocoa. Sterculiaceae [21]	*Guettarda acreana*, quina quina, yaki. Rubiaceae [20]		
	Moquilea tomentosa, pottery Tree. Rosaceae [14]		
	Syncarpia glomulifera, tur-Pentine tree. Myrtaceae [19]		

cient in the isolation of both varieties of *C. gattii* and *C. neoformans* var. *grubii* from the environment, thus, we corroborate the views of Staib [27]. However, it would be interesting to compare the efficacy of simplified Staib's niger seed agar without and with supplementing creatinine and KH_2PO_4 in the isolation of both varieties of *C. gattii* and *C. neoformans* var. *grubii*.

Till date, 23 tree species have been known to harbor *C. gattii* and *C. neoformans* var. *grubii* (Table 3). This number is likely to increase in future. The present study supports the recently emerging evidence that the natural habitat of both the varieties of *C. gattii* and *C. neoformans* var. *grubii* is not specific but instead is more generalized. More comprehensive studies are required in India as well as in other countries to further elucidate the natural occurrence of *C. gattii*, *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* and their serotypes in the environment.

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